

# Endotoxin exposure and atopic sensitization in adult pig farmers

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**Background:** Recent studies have reported a low prevalence of atopic sensitization and respiratory allergy in children growing up on farms.

**Objectives:** We sought to evaluate the dose-response relationship between endotoxin and atopic sensitization in adult farmers and to assess the effect on respiratory health outcomes.

**Methods:** Data on endotoxin exposure and serum IgE levels were available for 162 pig farmers from a cross-sectional case-control study, with case selection on the basis of respiratory symptoms. Exposure to endotoxin was modeled in detail, and respiratory health effects were assessed during a medical examination. Exploratory analysis was done by using nonparametric modeling and was followed by classical parametric regression.

**Results:** IgE to one or more common allergens was detected in sera from 28 (17%) farmers. The average (geometric mean) total serum IgE levels was 37 IU/mL (geometric SD, 4 IU/mL). A strong inverse relationship was found between endotoxin and sensitization to common allergens for exposures of 75 ng/m<sup>3</sup> or less, with an odds ratio of 0.03 (95% CI, 0.0-0.34) for a 2-fold increase in endotoxin. For endotoxin exposure of greater than 75 ng/m<sup>3</sup>, the association was weak (odds ratio, 1.2 [95% CI, 0.38-3.6]). No association was found between endotoxin exposure and total serum IgE levels. Endotoxin was associated with increased airway hyperresponsiveness to histamine and lower lung function in sensitized farmers, without evidence of a nonlinear relationship.

**Conclusions:** The prevalence of atopic sensitization in adult pig farmers is low. Endotoxin or related exposures might protect from sensitization, even in an adult working population exposed to high levels of endotoxin, but is a risk factor for increased airway hyperresponsiveness and low lung function. (*J Allergy Clin Immunol* 2005;115:797-802.)

**Key words:** Endotoxins, hypersensitivity, IgE, allergens, adult, occupational exposure, epidemiology

### Abbreviations used

AHR: Airway hyperresponsiveness

OR: Odds ratio

Recent epidemiologic studies have reported a considerably lower prevalence of atopic sensitization and symptoms of respiratory allergy in children, young adolescents, and even adults growing up on farms when compared with their peers living in the same rural areas.<sup>1</sup> Contact with livestock during the first year of life was identified as the factor that best explained the protective effect on atopic sensitization,<sup>2,3</sup> which is consistent with the view that early life is a critical period for initiation of allergic immune responses and asthma.<sup>4</sup> It has been speculated that respiratory exposure to endotoxin (particularly in livestock farming) might be important.<sup>5</sup> Several studies have shown that animal keeping is associated with exposure to high levels of bacterial endotoxin.<sup>6-8</sup> There are strong indications that endotoxin exposure might cause or aggravate respiratory symptoms,<sup>9-12</sup> but experimental studies have shown that timing might be crucial. Consequently, studies on the possible protective effect of endotoxin exposure have focused almost exclusively on early childhood exposure.<sup>13-16</sup>

Some studies have indicated that rural living<sup>17</sup> or being a farmer<sup>18</sup> is associated with a reduced risk of atopy and atopic disease in adulthood as well, and we have shown earlier that growing up on a farm and current farming were independently associated with a lower prevalence of atopic sensitization.<sup>19</sup> Only one study has investigated the separate effects of having been born on a farm and current endotoxin exposure.<sup>13</sup> Having been born on a farm was associated with a reduced risk of sensitization in school-age children, but there was an additional protective effect of current endotoxin exposure.

It is not clear whether adult farmers also have a reduced risk of atopic sensitization, whether this is associated with their endotoxin exposure, and whether this has an effect on respiratory health outcomes. We therefore analyzed these relationships in a study among pig farmers. Exposure to endotoxin was measured on more than one occasion and used to model average long-term exposure. Earlier analyses have shown that high endotoxin exposure was associated with increased respiratory symptoms and

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Supported by grant no. 99.33 from the Dutch Asthma Foundation (L. Portengen).

Received for publication November 11, 2003; revised November 29, 2004; accepted for publication November 30, 2004.

Available online February 10, 2005.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.11.046

airway hyperresponsiveness (AHR) to histamine and with lower lung function in sensitized farmers.<sup>20,21</sup> The relationship between endotoxin exposure and atopic sensitization was not studied in detail, although it was apparent that the prevalence of sensitization to common allergens in the population was low. We analyzed the relationship between exposure to endotoxin and atopic sensitization and assessed the effect on respiratory health outcomes.

## METHODS

### Population and health data

Data were from a cross-sectional survey in pig farmers conducted in the early 1990s. A detailed description of the design and methods of data collection have been reported previously.<sup>20,21</sup>

The population consisted of 194 pig farmers living in the 2 southeastern provinces of the Netherlands, which were selected from a group of 1133 male owners of pig farms who worked at least 5 hours per day in pig farming. Selection was based on chronic respiratory symptoms reported in the Dutch version of a self-administered shortened questionnaire on respiratory symptoms of the British Medical Research Council.<sup>22</sup> All farmers (n = 94) with more than one symptom of chronic cough, chronic phlegm, ever or frequent wheezing, shortness of breath, and chest tightness (asthma) were included, and a group of 100 control subjects was selected at random from the symptom-free farmers. In a subsequent medical survey held in winter 1990-1991, venous blood samples were taken for IgE analysis. The medical ethical committee of the University Nijmegen approved the study. Subjects received information about the trial and consented to participate in writing.

### IgE measurements

Sera were stored at  $-20^{\circ}\text{C}$  until IgE analysis. Total IgE and specific IgE antibodies to the common allergens house dust mite, grass pollen (mix of 2 species), birch pollen, and cat were measured by means of enzyme immunoassays.<sup>23</sup>

Allergen-specific IgE was assessed with 1:10 diluted sera in allergen-coated microwells. A serum was considered positive if the optical density at 492 nm exceeded the mean optical density + 3 SD of the reagent blank (no serum control). Total serum IgE levels were measured by means of sandwich enzyme immunoassay with sera diluted 1:10, 1:20, and 1:40, and the IgE standard for the Pharmacia CAP system was used as a calibration standard.<sup>23</sup>

### Lung function and AHR

Forced expiratory lung function measurements were conducted with a Vicatest V dry rolling seal spirometer (Mijnhardt, Bunnik, The Netherlands). Measurements were performed according to the lung function protocol of the European Community for Steel and Coal.<sup>24</sup>

Airway responsiveness was tested by using histamine provocation according to a modified procedure of the method of Cockcroft et al.<sup>25</sup> Histamine concentration ranged from 0.03 to 16 mg/mL. AHR was defined as a decrease in FEV<sub>1</sub> of at least 10% at a histamine concentration of 16 mg/mL or less.<sup>26</sup>

### Exposure measurements and modeling

Personal inhalable dust samples were taken twice: once in the summer and once in the winter. Dust was collected by using PAS6 sampling heads and 1- $\mu\text{m}$  Teflon filters (Millipore, Billerica, Mass) at an airflow of 2 L/min.<sup>6</sup> Samples were stored at  $-20^{\circ}\text{C}$  until extraction. Endotoxin was extracted in 0.05% (vol/vol) Tween 20 in pyrogen-free water and stored at  $-20^{\circ}\text{C}$  until analysis.<sup>27</sup> Endotoxin levels were measured with the Lymulus Amebocyte Test, according to procedures

described earlier.<sup>27</sup> Endotoxin units were converted to nanograms of endotoxin by using a factor of 0.1 ng/EU.

Endotoxin levels were expressed in nanograms per cubic meter. On the basis of the relationship between endotoxin concentration and farm characteristics and time spent on activities in pig farming during 2 full weeks, the long-term time-weighted average exposure to endotoxin was estimated.<sup>28</sup> Estimation was based on log-transformed exposure levels to standardize variance and obtain normally distributed residuals. The measurements in the summer and winter were used as independent observations because the correlation between them was low. The final model included outdoor temperature, 12 farm characteristics, and 8 activities in pig farming and explained 37% (adjusted  $R^2 = 33\%$ ) of the variation in log-transformed time-weighted average endotoxin exposure. In this article endotoxin exposure is defined as modeled individual long-term average exposure to endotoxin.

### Data analysis

IgE sensitization to common allergens was defined as a positive reaction to one or more common allergens. Endotoxin exposure and total IgE levels were best described by using a log-normal distribution. Total IgE levels were either log transformed or dichotomized by using 100 IU/mL as a cutoff level. For exploratory analysis, the relationship between log-transformed endotoxin exposure and sensitization to common allergens, (log-transformed) total IgE, and respiratory outcomes was studied by means of generalized additive modeling (smoothing) with PROC GAM (SAS for Windows version 8.0; SAS Institute, Cary, NC).<sup>29</sup> For dichotomous response variables, a logistic model was used, and smoothed curves were computed by using a logit-link function and transformed to prevalences by applying the inverse of the logit function. The smoothness of the function was determined by means of generalized cross-validation.<sup>30</sup>

On the basis of results from this analysis, log-transformed endotoxin exposure was used in multiple regression analysis by using more conventional parametric models. A *P* value of less than .05 was considered statistically significant.

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## RESULTS

### Subject characteristics, atopic sensitization, and total IgE levels

Complete data on endotoxin exposure, personal characteristics, and serology were available for 81 cases and 81 control subjects. Subject characteristics, IgE sensitization, and respiratory health outcomes in the study population stratified by case-control status are presented in Table I. Cases were somewhat older, smoked more often, and had been working with pigs for a longer time than control subjects.

Specific IgE to at least one of the 4 common allergens was detected in 28 (17%) farmers and was weakly associated with case-control status (odds ratio [OR], 1.4 [95% CI, 0.63-3.3]). Most sensitized farmers had IgE to house dust mite (61%) or grass pollen (36%); only 5 (18%) were sensitized to birch pollen, and none were sensitized to cat allergen. Four (14%) subjects were sensitized to more than one allergen. The average (geometric mean) total

serum IgE level was 37 IU/mL (geometric SD, 4 IU/mL) and was slightly higher in cases than in control subjects.

AHR was present in 50 (31%) of 159 farmers and was strongly associated with case-control status (OR, 4.1 [95% CI, 2.0-8.5]). Average lung function was slightly higher than predicted on the basis of age- and height-corrected reference values in control subjects, whereas FEV<sub>1</sub> was somewhat less than predicted in cases.

### Endotoxin exposure and allergic sensitization

Endotoxin exposure was similar for cases and control subjects and ranged from 36 to 316 ng/m<sup>3</sup> (Table I). No significant association was found between endotoxin exposure dichotomized at the median level of 101 ng/m<sup>3</sup> and specific sensitization (OR, 0.88 [95% CI, 0.34-2.3]) or increased levels (>100 IU/mL) of total IgE (OR, 1.2 [95% CI, 0.53-2.6]) in logistic regression analysis adjusting for age and current smoking, as reported earlier.<sup>21</sup>

In contrast, the OR for a 2-fold increase in exposure and sensitization to common allergens was 0.44 (95% CI, 0.19-0.98) when log-transformed endotoxin exposure was included as a continuous covariate. The shape of the dose-response relationship was studied in more detail by using nonparametric models (smoothing). Results from this analysis suggested a significant nonlinear relationship between endotoxin exposure and sensitization to one or more common allergens (Fig 1). Risk of sensitization strongly decreased with increasing exposure from a predicted 70% at the lowest exposure to 20% at an exposure of 75 ng/m<sup>3</sup> and remained virtually unchanged for even higher exposures. We therefore allowed for different regression coefficients for endotoxin exposure less than and greater than the level of 75 ng/m<sup>3</sup> in classical logistic regression analysis, including age, current smoking, and case-control status as potential confounders. This broken-stick model fitted the data significantly better than a model without a breakpoint (likelihood ratio  $\chi^2 = 5.8$ ,  $P = .02$ ). The results confirmed the strong inverse relationship between endotoxin exposure and sensitization to common allergens for exposures of up to 75 ng/m<sup>3</sup> with an estimated OR of 0.03 (95% CI, 0.0-0.34) for a 2-fold increase in endotoxin. For endotoxin exposure of greater than 75 ng/m<sup>3</sup>, the association was weak and not statistically significant (OR, 1.2 [95% CI, 0.38-3.6]). Inclusion of a familial history of atopy or disinfectant use as potential confounders had only minor effects on these estimates, and no evidence was found for effect modification by either case-control status or current smoking in a stratified analysis.

Inspection of the dose-response curve for endotoxin exposure and sensitization to individual allergens suggested that the effect of endotoxin was strongest for sensitization to pollen, whereas there was little effect on sensitization to house dust mite (Fig 2). The small number of sensitized subjects did not allow any further detailed analysis.

Only a weak positive association was found between log-transformed endotoxin exposure and log-transformed total IgE levels (estimated coefficient  $\beta$  [SE] = 0.19

**TABLE I.** Subject characteristics, IgE sensitization, and respiratory health outcomes in pig farmers with and without chronic respiratory symptoms

	Control subjects, n (%) / mean (SD)	Cases,* n (%) / mean (SD)
N	81	81
Age (y)	36 (9)	40 (10)†
Smoker	15 (19)	37 (46)†
Former smoker	25 (31)	25 (31)
Pack-years	7 (11)	15 (15)†
Years in pig farming	13 (8)	16 (8)†
Endotoxin exposure (ng/m <sup>3</sup> ), GM (GSD)	105 (1.4)	103 (1.5)
IgE to common allergens		
≥1	12 (15)	16 (20)
House dust mite	6 (7)	11 (14)
Grass pollen	6 (7)	4 (5)
Birch pollen	3 (4)	2 (2)
Cat dander	0 (0)	0 (0)
Total IgE ≥100 IU/mL	17 (21)	25 (31)
Total IgE, GM (GSD)	31 [4.0]	45 [3.9]†
AHR, n/N (%)	14/81 (17%)	36/78 (46%)†
FEV <sub>1</sub> (% predicted)	107 (14)	95 (17)†
FVC (% predicted)	112 (13)	104 (12)†

GM, Geometric mean; GSD, geometric SD; FVC, forced vital capacity.  
\*Cases reported at least 1 chronic respiratory symptom (cough, phlegm, shortness of breath, wheeze, or chest tightness).  
† $P < .05$  compared with control subjects.

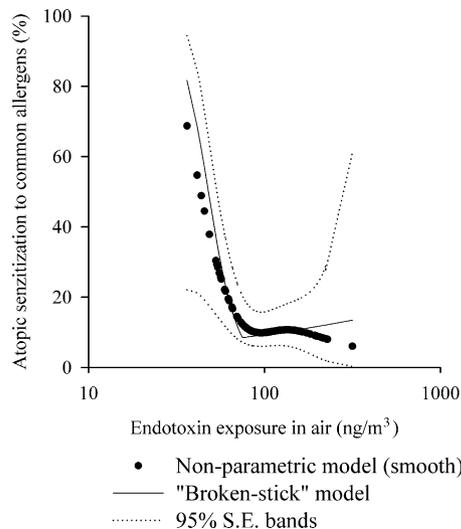
(0.30),  $P = .5$ ), with no evidence for a nonlinear relationship. No association was found between endotoxin exposure and increased levels of total IgE (>100 IU/mL) in logistic regression.

### Respiratory health effects

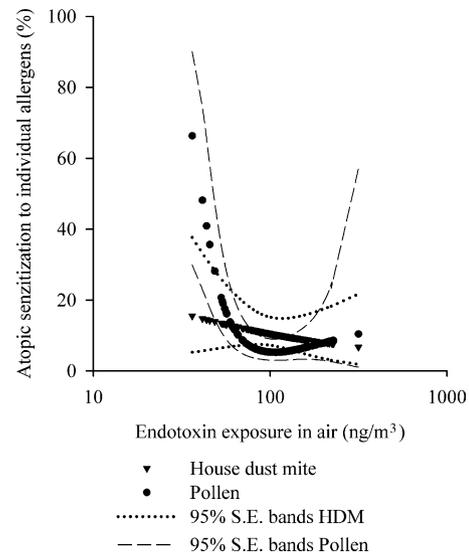
Relationships between endotoxin exposure and a number of respiratory health outcomes in this population have been studied earlier in detail by using logistic and linear regression analysis.<sup>20,21,31</sup> We used nonparametric models to investigate the shape of the dose-response relationship between endotoxin exposure and chronic respiratory symptoms, AHR, and lung function, adjusting for age, smoking habits, and standing height (lung function only). Endotoxin exposure was associated with increased AHR and lower FEV<sub>1</sub> but not with the presence of chronic respiratory symptoms (Fig 3). The relationship between endotoxin and forced vital capacity was very similar to that for endotoxin and FEV<sub>1</sub> and is therefore not shown. Associations were stronger for sensitized than nonsensitized farmers, although the interaction between endotoxin and sensitization was statistically significant only for AHR (Table II). No evidence was found for any nonlinear dose-response relationships.

### DISCUSSION

Only 17% of the full-time pig farmers in this study were sensitized to one or more common allergens. This is considerably lower than the prevalence of 32% reported for male subjects aged 20 to 70 years in the general Dutch



**FIG 1.** Inhalatory endotoxin exposure and IgE to common allergens in Dutch pig farmers: smoothed (cubic spline) plot with pointwise  $\pm 2$  SE bands and fitted broken-stick model.



**FIG 2.** Inhalatory endotoxin exposure and IgE to pollen and house dust mite in Dutch pig farmers: smoothed (cubic spline) plots with pointwise  $\pm 2$  SE bands.

population<sup>32</sup> and also lower than prevalences of 27% to 38% reported in studies in other occupational groups in which the same IgE assays were used.<sup>33-35</sup> Exposure to endotoxin was associated with a strongly reduced risk of sensitization for exposures of up to 75 ng/m<sup>3</sup> but did not seem to have an additional effect at higher exposures.

There was no indication that the inverse relationship between endotoxin and IgE sensitization had an effect on the relationship between endotoxin and respiratory health effects in this population, even though dose-response curves between endotoxin and respiratory outcomes were steeper in sensitized farmers. Dose-response curves for endotoxin and respiratory symptoms, AHR, and lung function all showed a monotonically increasing risk with higher endotoxin exposures, both in stratified and non-stratified analyses. This could be due to the low number of sensitized farmers in the study and the generally weak association between IgE sensitization and respiratory outcomes in this population.

Our results suggest that work-related exposure to endotoxin (or associated factors) rather than lifestyle factors explains the different prevalence of atopic sensitization in farmers and nonfarmers and that exposure to endotoxin protects against atopic sensitization, even in adults. Severe confounding by differences in lifestyle factors in this study seems unlikely because all subjects were full-time pig farmers from the same general geographic area. The correlation between lifestyle factors and occupational endotoxin exposure is therefore expected to be much lower than in studies comparing farmers and farm children with rural control subjects. Confounding by childhood farm exposures is also unlikely because virtually all farmers will have been born on a farm, and the correlation between occupational endotoxin exposure and early-life exposures is therefore expected to be low. Information regarding childhood exposure was not available for this

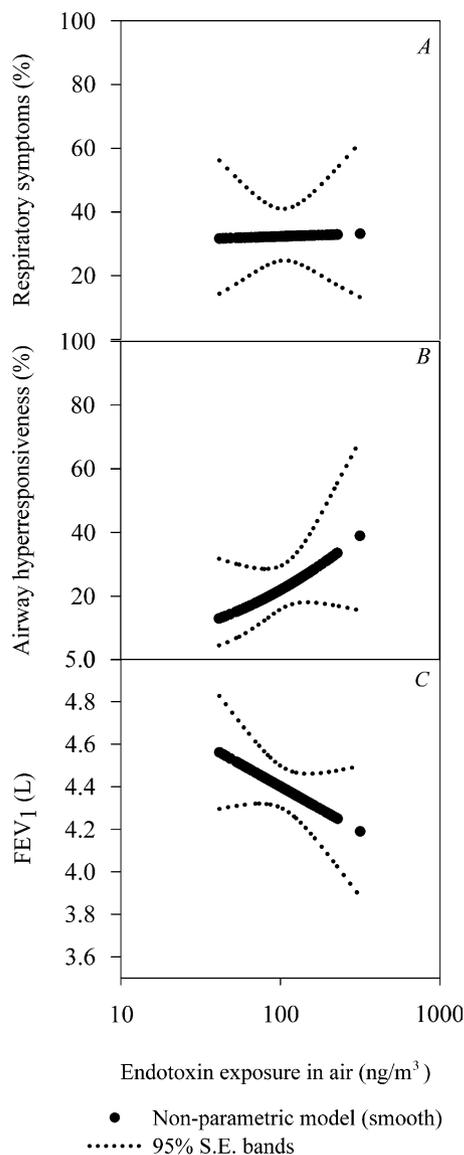
study, but more than 95% of pig farmers participating in a recent study in the same general geographic area indicated they had been born on a farm (not published).

Although lifestyle factors can probably be ruled out, endotoxin might still be a surrogate marker for exposure to other agents of microbial or animal origin.<sup>36</sup> The most important determinants of modeled exposure were activity patterns involving (close) contact with animals and flooring characteristics,<sup>28</sup> and these are probably not very specific for endotoxin.

Because of the cross-sectional design of the study, it is impossible to say whether the low prevalence of atopy in highly exposed farmers is a consequence of a reduced incidence or an increased remission of sensitization. The fact that the effect of endotoxin might be attributed to current exposure in adults suggests the latter process could be involved.

For individual allergens, endotoxin had a stronger effect on sensitization to grass pollen than on sensitization to house dust mite, which is consistent with the results from other studies in farm children and farming adults.<sup>18,37-39</sup> That we did not find an association between endotoxin exposure and total IgE levels could be considered inconsistent with an effect on atopic sensitization, but others have argued that total IgE levels are more strongly hereditary and less influenced by environmental factors.<sup>40</sup>

Major limitations of this study are the small population size and the fact that the study was designed to identify risk factors for chronic respiratory disease. Endotoxin exposure could be modeled for only 162 farmers, and half of these had been selected on the basis of rather general, but not work-related, respiratory symptoms. However, there was no evidence that the design had a major effect on the results because the relationship between endotoxin exposure and sensitization was similar for patients and control subjects. The allergen panel we used to define



**FIG 3.** Inhalatory endotoxin exposure and chronic respiratory symptoms (A), AHR (B), and FEV<sub>1</sub> (C) in Dutch pig farmers: smoothed (cubic spline) plots with pointwise  $\pm 2$  SE bands.

atopic sensitization did not include dog allergens. This is unlikely to have affected our results because sensitization to dog allergens is generally even less common than sensitization to cat allergens, and we did not find a single farmer with IgE to cat allergens.

The assay that was used was based on an in-house protocol. However, it had previously been validated against skin prick test results in a series of 116 children's serum samples.<sup>27</sup> Sensitivity and specificity of the enzyme immunoassay results as a predictor of skin prick test reactivity toward common allergens were greater than 80% to 90%. The assay has also been used extensively in other epidemiologic studies.<sup>33,34,41</sup>

Misclassification of exposure or IgE status could have occurred but has probably been nondifferential and would have led to underestimation of the exposure response

**TABLE II.** Association between endotoxin exposure and respiratory health effects in pig farmers

Outcome	Population	N	Effect	P value
<b>OR (95% CI)</b>				
Respiratory symptoms	All	162	1.0 (0.55 to 1.9)	.9
	Not sensitized	134	1.1 (0.54 to 2.3)	.8
	Sensitized	28	1.5 (0.33 to 6.9)	.6
<b>OR (95% CI)</b>				
AHR	All	158	1.6 (0.79 to 3.4)	.19
	Not sensitized	132	1.2 (0.56 to 2.7)	.6
	Sensitized	26	17 (1.3 to 227)	.03
<b><math>\beta</math> (95% CI)</b>				
FEV <sub>1</sub>	All	157	-127 (-313 to 60)	.18
	Not sensitized	130	-80 (-292 to 133)	.5
	Sensitized	27	-220 (-619 to 180)	.3

relationship. One of the goals of exposure modeling was to reduce misclassification by increasing the number of repeated exposure estimates per individual because pig farmers usually stick to a weekly working schedule, with considerable variation between days of the week.

Healthy worker selection could be a more serious problem because the relationship between endotoxin exposure and sensitization would be seriously biased when those with allergic symptoms (and IgE) avoid exposure to endotoxin. However, only one subject reporting (non-work-related) allergic symptoms was exposed at endotoxin levels of 75 ng/m<sup>3</sup> or less, and a strong relationship between endotoxin and sensitization was also found in those not reporting any symptoms.

Confounding by age, smoking habits, and case-control status has been taken into account in the present analysis, but other potential confounders, such as familial history of atopy, history of allergic symptoms in childhood, use of disinfectants, and number of years working with pigs, were all considered. From the difference between our and earlier reported findings,<sup>21</sup> it is clear that the cutoff value that is chosen for the logistic regression analysis is important. Our cutoff of 75 ng/m<sup>3</sup> was based on the results of an exploratory nonparametric model that clearly indicated a nonlinear relationship between endotoxin exposure and sensitization. However, use of simple logistic regression with log-transformed modeled endotoxin exposure as a continuous covariate also resulted in a very low estimated OR for endotoxin and sensitization to common allergens. Therefore the cutoff of 101 ng/m<sup>3</sup> that was chosen for the earlier analysis is clearly suboptimal.

In conclusion, average long-term endotoxin exposure was associated with a reduced prevalence of sensitization to common allergens in a highly exposed adult farming population but was a risk factor for increased AHR and lower lung function in sensitized farmers. There was no evidence that the lower risk of sensitization results in a lower risk of adverse respiratory health effects. The proinflammatory effects of endotoxin exposure might therefore outweigh the potential beneficial effect on allergic sensitization. Longitudinal studies in populations

with a high exposure to allergens and endotoxin are needed to investigate whether the protection from sensitization has an effect on the development or severity of respiratory symptoms in populations in which allergic respiratory disease is more common and whether endotoxin might reduce pre-existing sensitization.

We thank Professor James A. Deddens of the Department of Mathematics, University of Cincinnati, for his advice on evaluating nonlinear dose-response relationships.

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