ORIGINAL ARTICLE

Sensitisation to common allergens and respiratory symptoms in endotoxin exposed workers: a pooled analysis

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ABSTRACT

Objective To test the hypotheses that current endotoxin exposure is inversely associated with allergic sensitisation and positively associated with non-allergic respiratory diseases in four occupationally exposed populations using a standardised analytical approach.

Methods Data were pooled from four epidemiological studies including 3883 Dutch and Danish employees in veterinary medicine, agriculture and power plants using biofuel. Endotoxin exposure was estimated by quantitative job-exposure matrices specific for the study populations. Dose—response relationships between exposure, IgE-mediated sensitisation to common allergens and self-reported health symptoms were assessed using logistic regression and generalised additive modelling. Adjustments were made for study, age, sex, atopic predisposition, smoking habit and farm childhood. Heterogeneity was assessed by analysis stratified by study.

Results Current endotoxin exposure was dose-dependently associated with a reduced prevalence of allergic sensitisation (ORs of 0.92, 0.81 and 0.66 for low mediate, high mediate and high exposure) and hay fever (ORs of 1.16, 0.81 and 0.58). Endotoxin exposure was a risk factor for organic dust toxic syndrome, and levels above 100 EU/m^3 significantly increased the risk of chronic bronchitis ($p<0.0001$). Stratification by farm childhood showed no effect modification except for allergic sensitisation. Only among workers without a farm childhood, endotoxin exposure was inversely associated with allergic sensitisation. Heterogeneity was primarily present for biofuel workers.

Conclusions Occupational endotoxin exposure has a protective effect on allergic sensitisation and hay fever but increases the risk for organic dust toxic syndrome and chronic bronchitis. Endotoxin's protective effects are most clearly observed among agricultural workers.

What this paper adds

- Adult-onset endotoxin exposure is a well-known risk factor for respiratory disorders, but recent studies on farming populations suggest that it may also protect from allergic disease.
- Evidence on this dual effect of endotoxin exposure is limited, and whether the protective effects apply to other populations than farmers remains unclear.
- Occupational endotoxin exposure significantly increased the risk of chronic bronchitis and organic dust toxic syndrome symptoms.
- Inverse relationships between endotoxin and atopy and hay fever were found most clearly among workers with agricultural related exposures.

INTRODUCTION

Several adult population studies show that early life exposure to farming decreases the risk of allergic sensitisation and asthma throughout life.[1–4] This association is commonly attributed to high exposure of farm children to microbial agents of bacterial and fungal origin like endotoxin and β(1→3)-glucans.[5] In particular, exposure to such agents is speculated to stimulate the innate immune system, either by suppression of the atopic Th2 responses or by the induction of an increase in the shift from Th2 to the Th1 phenotype.[5] Evidence exists that this protective effect may not be limited to exposure during childhood. Studies in adult populations have indicated that farmers[6] and rural dwellers[1] have a lower risk of asthma and allergic sensitisation. These results were confirmed by studies using animal contact as a proxy for microbial exposure, demonstrating the lowest risk of allergic sensitisation in subjects combining farm childhood and farm animal contact in adulthood.[2 4 7] In addition, inverse associations between measured endotoxin exposure and atopy or allergic asthma were found in studies among Norwegian[8] and Dutch farmers,[9] and among agricultural industry workers.[10]

Beside its protective effects, bacterial endotoxin is well known for its pro-inflammatory capability. High occupational exposure to airborne endotoxin has long been associated with a number of acute and chronic health effects like organic dust toxic syndrome (ODTS), bronchial hyper-responsiveness, asthma-like symptoms, chest tightness, cough, shortness of breath, wheezing, chronic bronchitis, inflammation in the airways and accelerated lung function decline.[10–17] Interestingly, these adverse health effects were also found by studies in which inverse dose—response relationships between endotoxin exposure and allergic asthma or atopy were reported.[8–10 14] These intriguing findings indicate both a positive and negative role of endotoxin in the development of health effects in humans. Nevertheless, the information on this dual effect of endotoxin remains limited as half of the studies that reported it[8 9] did not consider farm exposures that occurred during childhood and were exclusively performed in farming populations where selection out of farming might also explain the observed inverse associations between endotoxin exposure and atopy.

In the present study, we pooled data from four studies of employees including veterinary students, farm apprentices, and biofuel and agricultural industry workers exposed to microbial exposures at work. We performed combined analysis and explored relationships between endotoxin exposure and allergic sensitisation, asthma and other respiratory diseases, taking early-life exposures to farming into consideration. Furthermore, we investigated whether dose–response relationships differed between the four subpopulations in order to explore the hypothesised extension of the effects of endotoxin exposure in other occupational groups than farmers.[10] The study was performed in the framework of the GABRIEL project (http://www.gabriel-fp6.org/).

MATERIALS AND METHODS

Study design

The present study is a pooled analysis of the baseline data from four studies from Denmark and The Netherlands: (1) a cross-sectional investigation that explored relationships between current endotoxin exposure and respiratory and allergic outcomes in a population of 525 farmers (participation rate 61%) and 376 workers in 23 agricultural processing companies (participation rate 90%) in The Netherlands;[10] (2) a Danish prospective cohort study (SUS) including 1964 farm apprentices (participation rate 79%) that explored the role of farm exposures on the development of atopy and respiratory diseases in young farmers;[3 18 19] (3) a cross-sectional study among Danish power plant workers that assessed relationships between bio-aerosol exposure, allergy and respiratory health;[17] this study included 94 power plant workers using straw (participation rate 75%) and 138 power plant workers using wood chip (participation rate 74%); (4) a cross-sectional study that addressed the effect of bio-aerosol exposure on the development of allergic and non-allergic respiratory diseases in veterinary students (the veterinarians' health study). The study population consisted of veterinary students at the Utrecht University participating in the study from June to October 2006 (n=901, participation rate 65%). More details on the design and methodology of the specific study are given in the online supplement A.

All four studies used detailed questionnaires on asthma, atopy, familial history of asthma and/or allergy, smoking and occupational history. The comparability between questionnaires was assessed based on the meaning and timing of questions referring to the same airway or atopic symptom or personal characteristic (see online supplement B, table S1, for details).

The questionnaire was followed by a comprehensive health investigation in all four studies. Allergic sensitisation against common inhalant allergens (pollen, house dust mite, cat, dog) was assessed by means of skin prick tests in the Danish studies and by serological testing of specific IgE using enzyme immunoassays[20] in the Dutch studies.

Pooled population

Questionnaire information was available for all 3998 subjects in the four studies. In addition, information on IgE-mediated sensitisation was available for 434 (342 processing workers, 92 farmers) agricultural industry workers, 641 veterinary students, 1959 farm apprentices and 200 (120 wood chip workers, 80 straw workers) biofuel workers. Twenty-one farm apprentices, 4 veterinary students and 46 biofuel workers without data on exposure as well as 1 biofuel and 15 agricultural industry workers aged >65 years were excluded from the analysis. A further 28 workers (10 farm apprentices, 9 agricultural industry and 9 biofuel workers) with incomplete data on potential confounders (farm childhood, age, familial history of allergic diseases or smoking status) were also removed resulting in 3170 and 3883 subjects available for statistical analysis with sensitisation and symptoms, respectively. Online supplement C, figure S1, represents a schematic overview of the pooling process.

Health outcome definitions

Asthma was defined as a positive response to any of the following questions: 'Are you currently taking any medicine for asthma?', 'Have you ever had asthma?', 'Have you sometimes had wheezing in the chest, during the last year?' and 'During the last 12 months have you sometimes been woken up with a feeling of tightness in your chest?' Chronic bronchitis was defined as 'coughing up phlegm almost daily, for 3 months in a row during the last year' and wheezing as 'at least one attack of wheezing during the last year'. A combination of self-reported pollen allergy accompanied by eye (itching or watery eyes) or nose (sneezing) symptoms or a positive answer to the question 'Have you ever had hay fever?' was used to define hay fever. Atopy was determined as elevated serum IgE levels or positive skin prick tests (the mean of the longest diameter and the midpoint orthogonal diameter of the weal >3 mm) to one or more of the following common allergens: pollen (grass or birch), house-dust mites, and cat and dog (details in online supplement B, table S1). Self-reported allergy was defined as self-reported allergic reactions (lung, nose and/or eye symptoms) against pollen, animal or house-dust allergens. An affirmative answer to the question 'Have you, during the past 12 months, had sudden episodes of flu-like symptoms such as fever, chills, malaise, muscle or joint pains, and felt completely well within 1–2 days?' was used as a proxy for ODTS episodes.

Exposure assessment

Information on the participant's occupational and exposure history including information on specialisations, tasks, areas and the duration of exposure (eg, hours per day or weeks per year) was available from the study questionnaires. In addition, endotoxin levels were available from more than 1200 personal and stationary measurements performed within the investigations of the Danish biofuel and the Dutch agricultural and veterinary studies and during the 15th year follow-up of the Danish SUS study.[10 17 18 21 22] The current personal endotoxin exposure was estimated for every worker by means of quantitative Job-Exposure Matrices developed from measurements and the available questionnaire information in each of the participating studies (for details, see online supplement D). For 535 farm apprentices, who had complete information on exposure but were considered non-exposed in the corresponding Job-Exposure Matrices, a background exposure concentration of 1 EU/m^3 was assumed based on levels reported in non-industrial occupational[23 24] and residential[25] indoor environments.

Data analysis

Data analysis was performed using SAS statistical software (V.9.2; SAS Institute) for Windows. The pooled study population was divided into four exposure categories (low, low mediate, high mediate and highly exposed). Earlier proposed exposure standards of 50 and 200 EU/m^3 for endotoxin exposure by the National Health Council and the Social and Economical Council of The Netherlands[12 26] along with the suggested 'no-effect level' of 100 ng/m^3 (~1000 EU/m^3)[27] were used as cut-off points. Differences in prevalence of diseases and characteristics between subgroups of the pooled study population were assessed with χ^2 tests. Kruskal–Wallis and Mann–Whitney tests were used for comparison of continuous variables. Associations between endotoxin exposure and health outcomes were assessed with logistic regression analysis (PROC LOGISTIC), using the lowest exposure group as reference. Potential confounders (atopic predisposition, gender, smoking, age and exposure to farming during childhood) were considered in the analysis. The reliability of the demonstrated associations and the heterogeneity across populations were investigated by adjustment for study and by stratified analyses. Effect modification by farm childhood and atopic status were assessed using interaction terms in the multiple regression models and by stratified analyses.

The linearity and the shape of the estimated associations in the main analysis were further tested by: (a) using endotoxin exposure as a continuous variable in the final multivariate logistic regression models and (b) generalised additive modelling (smoothing).[28] All analyses with endotoxin exposure as a continuous variable were performed on log-transformed endotoxin exposure concentrations. The statistical significance level was set at 5% (two-sided) for all the applied tests and models.

RESULTS

Basic characteristics of the four studies and the pooled population are shown in table 1. Overall, the median estimated current average endotoxin exposure was 219 EU/m^3 (range: 0.01–10 645 EU/m^3). Exposure differed significantly between study populations ($p<0.0001$) and the estimated levels ranged (median) from 14–10 645 EU/m^3 (219 EU/m^3) for Dutch farmers and agricultural industry workers to 1–1495 (215 EU/m^3), 3.2–749 (309 EU/m^3) and 0.01–294 EU/m^3 (3.4 EU/m^3) for farm apprentices, veterinary students and biofuel workers, respectively. In addition, the study populations also differed significantly in gender, farm childhood, smoking habits and atopic predisposition. Farm apprentices were younger and reported allergic and respiratory symptoms less frequently than other occupational groups. Most of the participants (75%) who reported ODTS symptoms were Dutch farmers and agricultural industry workers. The prevalence of atopy was highest among veterinary students.

Table 2 summarises the results of univariate and multiple logistic regression analysis between occupational endotoxin exposure and health outcomes of interest. Significant positive

Table 1 Basic characteristics of 3883 workers in the pooled population and by participating study

	The Netherlands		Denmark		
	Agricultural industry workers (n=877)*	Veterinary students (n=897)*	Farm apprentices (n=1933)*	Biofuel workers (n=176)*	Pooled population (N=3883)*
Demographics					
Age, years	43.6±10.5	23.7±3.5	19.2±2.6	47.6±8.6	27.0±12.2
Gender, female	155 (17.7)	722 (80.5)	229 (11.9)	7 (4.0)	1113 (28.7)
Farming environment during childhood†	509 (58.0)	90 (10.0)	854 (44.2)	63 (35.8)	1516 (39.0)
Atopic predisposition					
Asthma or allergy in parents or siblings	273 (31.1)	674 (75.1)	587 (30.4)	30 (17.1)	1564 (40.3)
Asthma or allergy in parents	147 (16.8)	496 (55.3)	350 (18.1)	16 (9.1)	1009 (26.0)
Asthma or allergy in siblings	181 (20.7)	509 (56.7)	346 (17.9)	16 (9.1)	1052 (27.1)
Smoking habits					
Past smoker	292 (33.3)	79 (8.8)	91 (4.7)	53 (30.1)	515 (13.3)
Current smoker	199 (22.7)	94 (10.5)	568 (29.4)	54 (30.7)	915 (23.6)
Smoking history, pack-years*	6.9±11.4	0.6±1.8	1.1±2.6	14.4±21.2	2.9±8.1
Endotoxin exposure					
EU/m^3, median (range)	219 (14–10 645)	309 (3–749)	215 (1–1495)	3 (0.01–294)	219 (0.01–10 645)
<50 EU/m^3	68 (7.8)	263 (29.3)	535 (27.7)	134 (76.1)	1000 (25.8)
50–200 EU/m^3	172 (19.6)	45 (5.0)	153 (7.9)	30 (17.1)	400 (10.3)
200–1000 EU/m^3	506 (57.7)	589 (65.7)	1076 (55.7)	12 (6.8)	2183 (56.2)
>1000 EU/m^3	131 (14.9)	0 (0)	169 (8.7)	0 (0)	300 (7.7)
Health symptoms					
Chronic bronchitis‡	64 (7.3)	51 (5.7)	62 (3.2)	11 (6.3)	188 (4.9)
Wheezing‡	101 (11.5)	100 (11.2)	172 (9.2)	30 (17.2)	403 (10.5)
Asthma§	156 (17.9)	187 (20.9)	245 (12.8)	40 (23.3)	628 (16.3)
ODTS‡	161 (18.6)	17 (1.9)	11 (0.6)	26 (14.9)	215 (5.6)
Hay fever§	94 (10.7)	177 (19.7)	186 (9.7)	36 (20.8)	493 (12.7)
Self-reported allergy§	155 (17.7)	252 (28.1)	151 (7.8)	34 (19.9)	592 (15.3)
Atopy¶	83 (19.4)**	157 (24.6)††	345 (17.9)	30 (17.4)	615 (19.4)

Demographic data and data on health symptoms are presented as n (%) or as mean±SD.
*Numbers may vary due to missing values in the health outcome variables.
†Born, raised or lived in a farm for at least a year until the age of 5.
‡Self-reported symptoms during the last 12 months.
§Self-reported symptoms at any point in life.
¶Determined by serum IgE (The Netherlands) or skin prick tests (Denmark) against pollen, house-dust mites, and cat and dog allergens.
**Based on information for 429 (91 farmers, 338 agricultural processing workers) participants.
††Based on information from 639 participants.
EU, endotoxin unit; ODTS, organic dust toxic syndrome.

Table 2 Univariate and multiple logistic regression results describing the association between occupational endotoxin exposure and respiratory and allergic disorders

Symptom and exposure group	Univariate model OR (95% CI)	Adjusted model* OR (95% CI)	p Value for trend*
Chronic bronchitis†			
Low	1.00	1.00	
Low mediate	1.34 (0.75 to 2.40)	1.38 (0.74 to 2.54)	<0.0001
High mediate	1.40 (0.94 to 2.07)	1.49 (0.97 to 2.31)	
High	3.66 (2.23 to 6.00)	4.11 (2.36 to 7.15)	
Wheezing†			
Low	1.00	1.00	
Low mediate	0.74 (0.49 to 1.13)	0.80 (0.51 to 1.24)	0.2040
High mediate	1.01 (0.79 to 1.29)	1.10 (0.83 to 1.45)	
High	1.69 (1.16 to 2.45)	1.72 (1.14 to 2.60)	
Asthma‡			
Low	1.00	1.00	
Low mediate	0.76 (0.55 to 1.07)	0.80 (0.56 to 1.14)	0.5676
High mediate	0.93 (0.76 to 1.14)	0.96 (0.77 to 1.20)	
High	1.42 (1.03 to 1.96)	1.52 (1.07 to 2.15)	
ODTS†			
Low	1.00	1.00	
Low mediate	3.30 (2.02 to 5.39)	1.68 (0.95 to 2.96)	0.0078
High mediate	1.71 (1.14 to 2.56)	1.80 (1.07 to 3.02)	
High	3.89 (2.34 to 6.46)	2.44 (1.30 to 4.60)	
Hay fever‡			
Low	1.00	1.00	
Low mediate	0.95 (0.69 to 1.32)	1.16 (0.82 to 1.63)	0.0177
High mediate	0.71 (0.57 to 0.88)	0.81 (0.64 to 1.02)	
High	0.45 (0.29 to 0.71)	0.58 (0.36 to 0.93)	
Self-reported allergy‡			
Low	1.00	1.00	
Low mediate	0.88 (0.65 to 1.20)	0.90 (0.64 to 1.25)	0.0018
High mediate	0.71 (0.58 to 0.87)	0.70 (0.56 to 0.87)	
High	0.60 (0.41 to 0.88)	0.72 (0.47 to 1.08)	
Atopy§			
Low	1.00	1.00	
Low mediate	0.90 (0.64 to 1.26)	0.92 (0.65 to 1.30)	0.0299
High mediate	0.83 (0.68 to 1.01)	0.81 (0.66 to 1.00)	
High	0.66 (0.45 to 0.97)	0.66 (0.44 to 0.99)	

*Results adjusted for gender, age, farm childhood, atopic predisposition, smoking habits and participating study.
†Self-reported symptoms during the last 12 months.
‡Self-reported symptoms at any point in life.
§Determined by serum IgE or skin prick tests against pollen, house-dust mites, and cat and dog allergens.
ODTS, organic dust toxic syndrome.

associations between endotoxin exposure, chronic bronchitis and ODTS were found. In contrast, endotoxin exposure was associated, in a dose-dependent manner, with a decreased prevalence of atopy, self-reported allergy and hay fever. Exposure to high levels of endotoxin was significantly associated with asthma and wheezing symptoms. Adjustment for confounders did not substantially change the associations between endotoxin exposure and the health outcomes (0% to 12% difference between crude and adjusted ORs), except for ODTS where the association became less prominent but remained statistically significant (OR (95% CI)=2.07 (1.24 to 3.46), 1.77 (1.16 to 2.70) and 3.20 (1.87 to 5.49) for the low mediate, high mediate and high exposure groups, respectively). Further analysis with adjustment for participating study barely attenuated the estimated ORs for most of the health outcomes.

Analysis of the data with non-parametric methods (smoothing) confirmed the demonstrated pooled associations for chronic bronchitis and hay fever (figure 1). A clear rise (table 2; p value for trend <0.0001) in risk for chronic bronchitis from an exposure of 100 EU/m^3 and higher was seen, whereas for hay fever, the prevalence steadily decreased with exposure levels exceeding the 50 EU/m^3. In addition, endotoxin levels above 20 EU/m^3 tended to decrease the risk of atopic sensitisation (online supplement E, figure S2). A similar but steeper association was found for self-reported allergy. Despite the overall low frequency of ODTS, its prevalence tended to increase gradually with elevated levels of endotoxin exposure. A weak increasing trend was seen for symptoms of wheeze (online supplement E, figure S2).

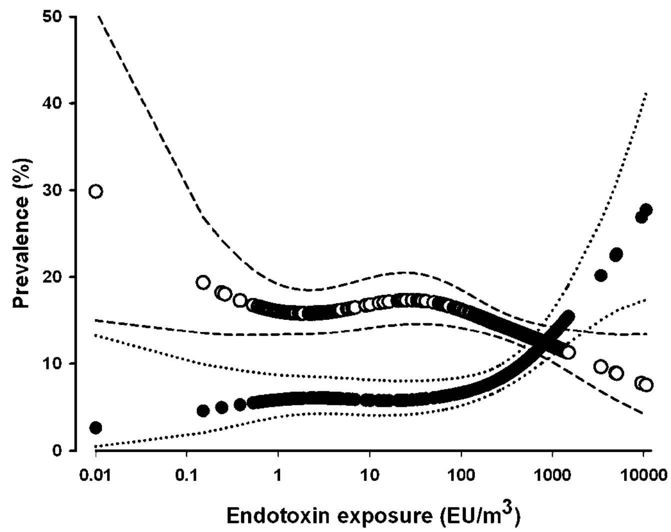

Figure 1 Smoothed relationships between endotoxin exposure, chronic bronchitis and hay fever for the pooled study population. Filled circle, chronic bronchitis; circle, hay fever; dotted line, ±95% CIs for chronic bronchitis; dashed line, ±95% CIs for hay fever. Results are adjusted for gender, age, farm childhood, atopic predisposition, smoking habits and participating study.

Study stratified analyses were undertaken to investigate possible heterogeneity between the involved populations (table 3). Overall, there were no major differences in the estimated relationships across the three subpopulations of agricultural industry workers, veterinary and farm apprentices for most of the health outcomes under investigation. However, the protective effects of endotoxin exposure on atopy and hay fever appeared to be more prominent in the Dutch agricultural study. Similarly, stronger dose–response relationships between endotoxin exposure, wheeze and asthma were seen among Dutch agricultural industry workers compared with veterinary students and farm apprentices. The subpopulation of biofuel workers showed the biggest differences compared with the other studies in the estimated associations for ODTS, atopy and hay fever. Nevertheless, sensitivity analysis by excluding Danish biofuel workers hardly changed associations (table 2) for any of the health outcomes under investigation (not shown).

We investigated whether associations between endotoxin exposure and asthma, wheeze and hay fever were modified by atopic status. Atopic and non-atopic subjects had approximately the same age, gender distribution, smoking habits and exposure distribution. But atopic workers were less likely to be exposed to farming during childhood (28.3% vs 39.3%) and had significantly more asthma (30.3% vs 12.1%), wheezing (21.1% vs 7.7%) and hay fever (43.0% vs 5.3%) symptoms than non-atopic workers.

Interestingly, in stratified multivariate analysis the prevalence of wheeze and asthma increased with exposure above

Table 3 Multiple logistic regression results describing the association between endotoxin exposure and health outcome on each of the involved studies (results are adjusted for farm childhood, gender, age (continuous), atopic predisposition and smoking habits)

Symptom and exposure group	Agricultural industry workers OR (95% CI)	Veterinary students OR (95% CI)	Farm apprentices OR (95% CI)	Biofuel workers OR (95% CI)
Chronic bronchitis*				
Low	1.00	1.00	1.00	1.00
Low mediate	1.80 (0.37 to 8.82)	1.66 (0.51 to 5.44)	1.15 (0.31 to 4.21)	11.05 (1.27 to 96.35)†
High mediate	2.65 (0.61 to 11.43)	1.09 (0.56 to 2.15)	1.37 (0.69 to 2.72)	8.44 (0.49 to 145.09)†
High	6.04 (1.35 to 27.14)		3.34 (1.48 to 7.53)	
Wheezing*				
Low	1.00	1.00	1.00	1.00
Low mediate	1.81 (0.48 to 6.81)	0.31 (0.07 to 1.37)	1.02 (0.50 to 2.08)	1.78 (0.62 to 5.09)
High mediate	3.32 (0.98 to 11.25)	0.79 (0.50 to 1.24)	1.01 (0.68 to 1.49)	5.09 (1.28 to 20.24)
High	5.92 (1.67 to 20.99)		1.14 (0.64 to 2.05)	
Asthma‡				
Low	1.00	1.00	1.00	1.00
Low mediate	0.84 (0.37 to 1.87)	0.96 (0.43 to 2.14)	0.80 (0.44 to 1.48)	1.32 (0.50 to 3.49)
High mediate	1.26 (0.62 to 2.56)	0.91 (0.64 to 1.31)	0.87 (0.63 to 1.20)	3.60 (0.93 to 13.94)
High	1.91 (0.88 to 4.14)		1.24 (0.76 to 2.01)	
ODTS*				
Low	1.00	1.00	1.00	1.00
Low mediate	1.45 (0.64 to 3.30)	x§	7.31 (0.64 to 83.09)¶	2.59 (0.95 to 7.05)
High mediate	1.80 (0.85 to 3.82)	1.45 (0.46 to 4.61)¶	2.21 (0.26 to 19.14)¶	0.77 (0.09 to 6.82)
High	2.17 (0.95 to 4.95)		6.28 (0.61 to 64.82)¶	
Hay fever‡				
Low	1.00	1.00	1.00	1.00
Low mediate	0.94 (0.45 to 1.96)	0.98 (0.44 to 2.20)	1.19 (0.66 to 2.12)	0.68 (0.21 to 2.24)
High mediate	0.44 (0.22 to 0.88)	0.92 (0.64 to 1.33)	0.84 (0.59 to 1.20)	1.61 (0.36 to 7.08)
High	0.26 (0.10 to 0.67)		0.66 (0.36 to 1.22)	
Self-reported allergy‡				
Low	1.00	1.00	1.00	1.00
Low mediate	1.10 (0.56 to 2.18)	0.63 (0.30 to 1.32)	1.11 (0.58 to 2.11)	0.34 (0.09 to 1.30)
High mediate	0.71 (0.38 to 1.34)	0.68 (0.49 to 0.94)	0.78 (0.53 to 1.15)	0.55 (0.06 to 5.07)
High	0.72 (0.34 to 1.50)		0.74 (0.39 to 1.41)	
Atopy**				
Low	1.00	1.00	1.00	1.00
Low mediate	0.73 (0.32 to 1.70)	0.71 (0.27 to 1.88)	0.86 (0.54 to 1.39)	0.83 (0.25 to 2.82)
High mediate	0.35 (0.17 to 0.69)	0.78 (0.52 to 1.17)	0.90 (0.69 to 1.18)	1.36 (0.24 to 7.79)
High	0.25 (0.10 to 0.61)		0.75 (0.46 to 1.20)	

*Self-reported symptoms during the last 12 months.
†Calculated only for males.
‡Self-reported symptoms at any point in life.
§Not calculated due to infinitive likelihood.
¶Calculated only for current and non-smokers.
**Determined by serum IgE or skin prick tests against pollen, house-dust mites, and cat and dog allergens.
ODTS, organic dust toxic syndrome.

200 EU/m^3 among non-atopic workers and not in atopic workers (table 4). In addition, the previously demonstrated inverse association between endotoxin exposure and hay fever was present only among atopic subjects. However, all p values for the interaction between endotoxin exposure and atopy were >0.05 (ie, there were no formal interactions). Atopic status was available for only 18% (91 subjects) of the subpopulation of farmers in the Dutch agricultural industry study. A sensitivity analysis excluding the Dutch farmers' population from the analysis had only a small influence on the estimated relationships for both atopic and non-atopic subjects (not shown).

Univariate analysis showed a clear protective effect of farming exposure in childhood for atopy, self-reported allergy, hay fever, asthma and wheeze, with ORs (95% CI) of 0.61 (0.50 to 0.74), 0.49 (0.40 to 0.59), 0.50 (0.40 to 0.62), 0.61 (0.51 to 0.73) and 0.62 (0.50 to 0.78), respectively. Adjustment for potential confounders and study did not considerably affect the estimated relationships for farm childhood and the respective health end points, which remained strong and significant. Associations were not confounded by current exposure to endotoxin either (see online supplement F, table S2, for details).

Stratification of the population by farm childhood showed no evidence of effect modification for asthma, wheeze, self-reported allergy, hay fever and chronic bronchitis (p value for interaction >0.2). However, the relationship between endotoxin exposure and atopy was dependent on the presence of farm exposures in early life (p value for interaction =0.0086). In workers with a farm childhood, no association was found between endotoxin exposure and atopy (OR (95% CI)=0.90 (0.47 to 1.75), 1.31 (0.86 to 2.00) and 1.49 (0.73 to 3.05) for the low mediate, high mediate and high exposure groups, respectively). In contrast, a negative dose-dependent trend between endotoxin exposure and atopy was found in workers without a farm childhood (OR (95% CI)=0.98 (0.65 to 1.49), 0.68 (0.53 to 0.87) and 0.48 (0.30 to 0.79) for the low mediate, high mediate and high exposure groups, respectively).

Table 4 Logistic regression analysis stratified by atopic status describing the associations between endotoxin exposure and the health symptoms of interest

Symptom and exposure group	Non-atopic workers (n=2555) OR (95% CI)	Atopic workers (n=615) OR (95% CI)
Wheezing*		
Low	1.00	1.00
Low mediate	0.96 (0.49 to 1.88)	1.45 (0.69 to 3.05)
High mediate	1.25 (0.83 to 1.87)	1.03 (0.64 to 1.66)
High	2.04 (1.17 to 3.55)	0.80 (0.30 to 2.11)
Asthma†		
Low	1.00	1.00
Low mediate	0.75 (0.43 to 1.30)	1.52 (0.79 to 2.95)
High mediate	1.03 (0.76 to 1.41)	0.99 (0.65 to 1.50)
High	1.99 (1.26 to 3.12)	0.84 (0.37 to 1.94)
Hay fever†		
Low	1.00	1.00
Low mediate	1.37 (0.72 to 2.62)	1.28 (0.68 to 2.41)
High mediate	1.01 (0.65 to 1.56)	0.76 (0.52 to 1.11)
High	1.00 (0.46 to 2.20)	0.31 (0.13 to 0.74)

ORs are adjusted for gender, age, farm childhood, atopic predisposition and smoking habits and participating study.
*Self-reported symptoms during the last 12 months.
†Self-reported symptoms at any point in life.

DISCUSSION

In this study, we used pooled health data from four epidemiological studies (veterinary students, farm apprentices, and biofuel and agricultural industry workers) and exposure estimates based on measurements to investigate whether and how endotoxin exposure predicts the likelihood of allergic sensitisation and airway disease. We found significant, inverse dose−response relationships between endotoxin exposure, hay fever and atopy, while current endotoxin exposure was associated with an increased prevalence of chronic bronchitis and ODTS. A farm childhood was a protective factor for hay fever and asthma symptoms, independent of current exposure to endotoxin. However, current endotoxin exposure showed an inverse dose−response relationship with allergic sensitisation only in workers without a farm childhood.

Endotoxin exposure, farm childhood and atopic sensitisation

Our results confirm the previously demonstrated[9 10 14] protective effect of current endotoxin exposure in adulthood by showing clear inverse relationships between occupational endotoxin exposure and atopy and hay fever. Like previous studies suggesting that the protective effect of early childhood exposure to farming on atopic diseases is persistent in adulthood,[1−4] we found significantly less atopy and asthma symptoms in workers with a farm childhood than without a farm childhood. These associations were not confounded by the level of current exposure, and farm childhood did not significantly modify the effect of current endotoxin exposure on hay fever. On the other hand, the prevalence of atopic sensitisation was only inversely related to current endotoxin exposure in workers who did not live on a farm during childhood. These findings further support the hypothesised protective effect of adult-onset endotoxin exposure on allergic disorders. The absence of an association among workers with a farm childhood may be due to the very low prevalence of atopic sensitisation in this particular group.[14] Yet the use of farm childhood as surrogate for early life exposures can be prone to misclassification and is short in ability to classify intensity and type of exposure. A recent multicentre European study on children showed that atopic and asthma responses can vary depending on type of farming and applied farming practices.[29] Therefore, studies with additional measured early life exposures and with follow-up of health and exposure of workers since commencement of current exposures are of particular interest in revealing whether early-life or current-onset exposure is of highest relevance.

Similar trends were found for study-specific endotoxin-atopy and endotoxin-hay fever associations in the subpopulations of Dutch farmers, veterinary students and farm apprentices. These three populations are all closely related to agriculture (veterinary students handle farm animals as well and those are the ones with the highest exposure levels) suggesting that agricultural related exposures is of importance. However, our population of biofuel workers, the only non-agricultural related, is relatively small and lower exposed compared with the other occupational groups. Further studies with larger non-agricultural related populations and with more comparable exposure levels to those found in agricultural environments are needed in order to confirm this finding.

Endotoxin exposure, chronic bronchitis and ODTS

We found endotoxin exposure to be a dose-dependent risk factor for both ODTS and chronic bronchitis from exposure levels of 100 EU/m^3 and higher. Smit and colleagues in two recent studies among Dutch agriculture seed[15] and wastewater[30] workers showed high occupational exposure to endotoxin to significantly induce ODTS-like symptoms. Elevated endotoxin exposure was found to significantly increase the likelihood of chronic bronchitis and cough with phlegm symptoms among Norwegian[16] and Dutch[10] farmers, respectively.

Approximately 46% of the participants with self-reported chronic bronchitis also reported asthma symptoms. The prevalence of smoking was relatively similar (39.9% vs 33.6%) in persons with chronic bronchitis and persons with asthma symptoms; but asthmatic persons with chronic bronchitis were more likely to be smokers than persons with only chronic bronchitis or asthma symptoms (53% vs 28% vs 30%, respectively). Asthmatic subjects with chronic bronchitis had a longer (7 vs 3.6 pack-years) smoking history than asthmatic subjects without chronic phlegm symptoms. Exclusion of asthmatic cases from the analysis did not affect the relationship between endotoxin exposure and chronic bronchitis which remained positive and significant (OR (95% CI)=1.21 (1.08 to 1.35), p value for trend 0.0007). Thus, the dose-dependent association between endotoxin exposure and chronic bronchitis is unlikely to be due to misclassification between cases of asthma and chronic bronchitis.

Endotoxin exposure, atopic and non-atopic asthma

A Norwegian study in farmers that used personal exposure measurements [8] showed no association between asthma per se and endotoxin exposure, but after stratification for atopic status endotoxin exposure was positively associated with non-atopic asthma and negatively with atopic asthma. In our analysis, we were also unable to find clear patterns in the relationships between endotoxin exposure, asthma per se and wheezing; but when trying to confirm the presence of the two asthma phenotypes current endotoxin levels above 1000 EU/m^3 appeared to increase the prevalence of both asthma and wheezing in non-atopic workers. Nevertheless, there were no clear inverse dose−response relationships between endotoxin and atopic wheeze, and the patterns were similar even in further analysis using doctor-diagnosed asthma (at any point in life) as an end point (not shown). This lack of association in atopic wheezing participants is difficult to understand. It could be due to differences in the nature (ie, type of endotoxin, other organic dust exposures) and level of exposure, or it could be a result of methodological and analytical differences (ie, differences in the exposure assessment strategies and cut-off levels and in the asthma and atopy definitions) between the two studies. The combined skin prick and blood test definition of atopy that we used may also have an impact on the result. The lower sensitivity of the IgE test, compared with the skin prick test,[20] could have underestimated the prevalence of atopic sensitisation, and thereby attenuated the endotoxin—wheeze relationships among atopic workers. Nevertheless, a major effect of such bias seems unlikely; when we stratified by method of atopy assessment, we found no systematic differences in the exposure—response relationships for hay fever and self-reported allergy (see online supplement G, table S3).

Heterogeneity across studies

A major concern in pooled analysis is the heterogeneity of the estimates between the involved studies.[31] Indications for heterogeneity existed mainly for Danish biofuel workers. However, the pooled estimates were not considerably affected by the exclusion of the specific study population and adjustment for study had only a small influence on the estimated ORs for the pooled population (table 2). Furthermore, the results were similar in separate analyses with adjustment and stratification by country (not shown). Thus, although present, heterogeneity between the involved studies is unlikely to have substantially influenced the results.

Exposure misclassification

A previous exposure assessment study in biofuel plants showed that personal exposure levels are higher than levels measured by stationary sampling.[32] The use of stationary levels in the exposure estimations for biofuel workers most likely resulted in an underestimation of their personal exposure levels. This, probably, also reflects to the lower than background exposure levels (0.1 vs 1 EU/m^3) estimated within the specific population. However, biofuel workers accounted for only 5% of the total population of the present study, and, as discussed in the results section, their exclusion did not considerably affect the results of the main analysis.

It could be argued that the estimation of exposure based on endotoxin levels obtained from recent measurements does not accurately represent the exposure of the baseline SUS population at the early 1990s. The Danish primary agriculture sector underwent major structural and technological changes through the last 20 years. However, the currently measured endotoxin concentrations for pig, poultry and dairy farmers in the SUS study are comparable with the personal levels reported from studies in Dutch pig[33] and American poultry[34] and dairy cattle[35] farmers performed during the early 1990s. In order to check for the possibility of misclassification in the specific study we re-analysed the data. We used the livestock endotoxin exposure levels obtained from stationary measurements within the Danish part of a large European exposure assessment study[36] conducted in the same time period as the 'SUS' study along with the external field work measurements from the Dutch agricultural industry study.[10] The estimated trends in the exposure response relationships for the pooled and the SUS study populations remained essentially similar to the currently presented ones (not shown) despite that the use of external exposure levels resulted in differences in the exposure estimates. Although the possibility of exposure misclassification cannot be completely excluded we have no reason to believe that it has been differential to the health outcome.

Healthy worker effect

Because of its cross-sectional design, the present study is prone to the occurrence of both hire and survivor healthy worker effects.[37] However, the protective effect of current endotoxin exposure on hay fever was independent of the occurrence of allergic diseases in the family suggesting a limited impact of hire selection effects through multiple generations. The occurrence of survivor effects among Dutch agriculture industry workers has been addressed in an earlier publication.[14] Inverse associations between endotoxin exposure and IgE-mediated sensitisation against grass pollen were found also among persons without self-reported allergic symptoms indicating the absence of self-selection on the specific population. In addition, the subpopulations of farm apprentices and veterinary students consisted of individuals who only recently started their working careers. These young populations accounted for 73% of our study population and most of them (67%) were not exposed to farming during childhood. Exposure-dependent selection or survivor effects are unlikely to have occurred in these subpopulations, due to the short time interval that they have been exposed to endotoxin. The homogeneity in the estimated associations between endotoxin exposure and hay fever on these subpopulations further supports the reliability of the pooled estimates. Thus, a healthy worker effect is unlikely to have a major impact on the results of the present study.

Conclusion

In conclusion, the present pooled study is one of very few studies that used quantified exposure estimates in order to assess dose—response relationships between current endotoxin exposure, atopy and respiratory symptoms and diseases among adults. Furthermore, it enabled the assessment of differences in exposure—response relationships across a variety of occupationally exposed populations using a standardised approach that minimised existing methodological differences. Its results confirm earlier published associations and further suggest that the protective effects of endotoxin might be stronger when the exposure is agricultural related.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Ethics Committee for Aarhus County and the institutional ethics committee of the University of Utrecht (University Medical Center Utrecht, The Netherlands) for the participating Danish and Dutch studies, respectively.

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