Airborne Endotoxin Is Associated with Respiratory Illness in the First 2 Years of Life

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Endotoxins are lipopolysaccharide components of the outer membranes of gram-negative bacteria. Endotoxin has been implicated in byssinosis, organic dust toxic syndrome, and illness in swine confined animal feeding operations (Douwes et al. 2002). Endotoxin in settled dust in residential environments has been associated with an increase in asthma symptoms, asthma medications, and reductions in lung function in those with atopy or asthma (Douwes et al. 2000; Gehring et al. 2001a; Michel et al. 1991, 1996; Park et al. 2001a; Rizzo et al. 1997). Despite these adverse effects, early exposure may reduce future allergies and asthma (Lapa e Silva et al. 2000; Litonjua et al. 2002; Reed and Milton 2001; Von Ehrenstein et al. 2000; Von Mutius et al. 2000). Most studies were of adults or school-age children, with two focusing on infants. In the present study we examined the association between airborne endotoxin and the incidence of respiratory illnesses in children during the first 2 years of life. We accounted for exposure to a potential confounder, indoor fungus, which has been associated with respiratory symptoms and may be associated with the presence of indoor endotoxin (Gehring et al. 2001b; Verhoeff and Burge 2004).

Materials and Methods

Study design. Data for the present study were abstracted from an ongoing study of the influence of indoor environmental factors on respiratory illness during the first 2 years of life. The study began in 1997 in the province of Prince Edward Island, Canada, which has a population of approximately 150,000. The study was approved by the ethics review boards of the Ottawa Hospital and the Health Protection Branch of the Canadian government. Recruitment occurred during the late autumn and winter (cold season) of each year when the ground was frozen. Because of resource constraints, we recruited approximately 60 consecutive newborns each year. All physicians who practice obstetrics in the province participated in recruitment. Women in the third trimester of pregnancy received letters from the physicians’ offices describing the study and requesting participation. Interested women were contacted by telephone to obtain informed consent. Excluded from the study were babies born > 4 weeks premature, those with neonatal respiratory difficulties requiring prolonged hospitalization at birth, and those whose families expected to change residence within 2 years of birth. Only one child per household was studied. Baseline information was obtained on sociodemographics and family history. The participating parents maintained a daily symptom diary from birth until 2 years or until the study ended, on large multipurpose calendars. Each study family was phoned twice monthly to document information from the diary. If parents had omitted recording symptoms on a daily basis, they provided information for the previous 2 weeks based on recall. Parental reporting of child care attendance was also recorded every 2 weeks.

Definition of respiratory illness. We adapted the method of Samet et al. (1992) to define a respiratory illness episode, the purpose being to identify discrete acute illnesses as opposed to persistent ongoing symptoms, such as a chronically runny nose. We defined the beginning of an illness episode as 2 consecutive days with any one of the four following symptoms: stuffy nose, cough, wheeze, and shortness of breath. The illness episode starts on the first of these 2 consecutive days and ends when there are 2 consecutive days without any of these symptoms, the last day of the illness episode being the last day with a symptom.

The primary outcome of interest was the number of illness episodes prorated on an annual basis (number multiplied by 365/days of observation). A secondary outcome measure, illness days, was defined as the sum of all days occurring within illness episodes, also prorated on an annual basis. For example, if a child had two illness episodes each lasting 3 days, six illness days would be assigned. If a child had two illness episodes each lasting 5 days, 10 illness days would be assigned.

Air sampling for endotoxin and ergosterol. Sampling was done within the first year of birth, and for 81%, within the first 4 months. Air from the child’s bedroom was sampled for both endotoxin and ergosterol through a three-piece cartridge equipped with a polycarbonate filter for approximately 5–7 days with a Buck model SS sampling pump (AP Buck, Orlando, FL, USA) calibrated at 2 L/min at the beginning and end of the sampling. Forty-eight hours after the pump was installed, the flow rate was checked to ensure it was within 5% of the initial reading. Very high dust concentrations can clog the filter and reduce the pump flow. If this happened, the pump was stopped and air endotoxin concentration was calculated based on the reduced sampling time. The total volume of air sampled ranged from 6.0 to 23.9 m3, limited by the need to maintain an acceptable flow rate. Once collected, the cartridges and filters were sealed in new plastic bags and stored at room temperature under dry conditions.

For endotoxin analysis, the filters were extracted with deionized water (LRW,
endotoxin. A statistical analysis was performed to determine if the model with variables endotoxin, year of home sampling, temperature, age, and mean hour per week that the baby was cared for more than 1 day a week outside the home, percentage of postnatal interviews in which the baby was breast-fed, income, and categorized percentage of postnatal interviews where smokers were declared in home.

A potential confounder was defined as a variable (Tables 1 and 2) that, if added to the model, would change the parameter \( \beta \) of the natural logarithm of endotoxin by > 10%. No confounders were found for the illness episodes model. The residuals from the regression equation were normally distributed (Shapiro-Wilk statistic = 0.9932, \( p = 0.1362 \)). We also examined the homogeneity of variance assumption, and the chart of residuals against predicted values showed no particular pattern. Interactions biologically plausible were also tested, and none were found to be statistically significant at the 5% level. We found no evidence of interaction between allergies or asthma in parents and endotoxin. Careful examination of each of the partial residual plots (i.e., the component-plus-residual plot) did not reveal any sign of nonlinearity in the relationship between illnesses and air endotoxin. Further, adding a square term for endotoxin did not improve significantly the \( R^2 \) of the model.

The endotoxin measurement was made only at the beginning of the 2-year follow-up. To determine the robustness of the endotoxin–illness association, we measured it at several time points between the initial endotoxin

| Characteristic | Overall | 1st tercile | 2nd tercile | 3rd tercile | \( p \)-Value* |
|---------------|---------|-------------|-------------|-------------|------------|
| Male sex      | 167 (50.3) | 53 (47.8) | 54 (49.1) | 60 (54.1) | 0.61       |
| Parent with asthma or allergies | 174 (52.7) | 64 (57.7) | 50 (45.9) | 60 (54.6) | 0.19       |
| Parent with university education | 197 (59.3) | 66 (59.5) | 64 (59.2) | 67 (60.4) | 0.95       |
| Family income |                      |              |              |              | 0.61       |
| < $30,000   | 67 (20.2) | 19 (17.1) | 20 (18.2) | 28 (25.2) |           |
| $30,000–49,999 | 114 (34.3) | 39 (35.1) | 39 (35.5) | 36 (32.4) |           |
| ≥ $50,000  | 151 (45.5) | 53 (47.8) | 51 (46.4) | 47 (42.3) |           |
| Environment  |                      |              |              |              | 0.70       |
| Furry or feathered pets |               |              |              |              |            |
| Never        | 86 (25.9) | 33 (29.7) | 24 (21.8) | 29 (26.1) |           |
| Sometimes    | 126 (38.0) | 42 (37.8) | 42 (38.2) | 42 (37.8) |           |
| Always       | 120 (36.1) | 36 (32.4) | 44 (40.0) | 40 (36.0) |           |
| Exposure to smoke |       |              |              |              | 0.73       |
| Low          | 114 (34.3) | 33 (29.7) | 40 (36.4) | 41 (36.9) |           |
| Medium       | 110 (33.1) | 40 (36.6) | 33 (30.0) | 37 (33.3) |           |
| High         | 108 (32.5) | 38 (34.2) | 37 (33.6) | 33 (29.7) |           |
| Year tested  |                      |              |              |              | <0.0001    |
| 1998   | 52 (15.7) | 9 (8.1) | 15 (13.6) | 28 (25.2) |           |
| 1999   | 53 (16.0) | 8 (7.2) | 19 (17.3) | 26 (23.4) |           |
| 2000   | 45 (13.6) | 21 (18.9) | 14 (12.7) | 10 (9.0) |           |
| 2001   | 58 (17.5) | 36 (32.4) | 13 (11.8) | 9 (8.1) |           |
| 2002   | 58 (17.5) | 24 (21.6) | 14 (12.7) | 20 (18.0) |           |
| 2003   | 60 (18.9) | 13 (11.7) | 35 (31.8) | 18 (16.2) |           |

*\( p \)-Value of the Pearson chi-square test of association between the characteristic and endotoxin. *There are two missing values for parent with asthma or allergies.
measurement and symptom assessment. We would assume that a true causal association would remain stable or weaken over time. If the association increased or fluctuated randomly with time of follow-up, this would reduce the probability of a causal association. We measured the results from 90-, 180-, 270-, 360-, 450-, 540-, 630-, and 720-day windows around the time of endotoxin sampling. The regression model obtained previously for a 2-year period was applied to each of these windows. The β-coefficient for the effect of the natural logarithm of endotoxin on illness episodes and total illness days along with its 95% confidence interval were graphed against the size of the window.

Results

The characteristics of the 332 children, overall and stratified by bedroom airborne endotoxin level, are presented in Tables 1 and 2. Of the categorical variables (Table 1), only year of testing was significantly associated with endotoxin, with no secular trends (p = 0.0001). The pets variable was not associated with endotoxin concentrations. For dogs, the geometric mean pets variable was not associated with endotoxin episodes and total illness days, respectively (p = 0.01). The annualized number of respiratory illness episodes and total days of illness episodes were positively related to endotoxin at p = 0.13 and p = 0.07, respectively (Table 3). All of the individual respiratory symptoms were greater in the higher compared with the lower endotoxin group, but only the incidence of wheeze reached statistical significance, being a relative 248% greater in the higher compared with the lower endotoxin group (p = 0.01).

The unadjusted Pearson correlation coefficients between log-transformed endotoxin and illness episodes and illness days were 0.105 (p = 0.056) and 0.106 (p = 0.053), respectively. The association for number of days with wheeze was 0.271 (p < 0.0001), but other individual variables were not significant at p = 0.05. The adjusted associations for illness episodes and total illness days were highly significant (Tables 4 and 5). The multiple linear regression model for illness episodes resulted in a β-coefficient of 0.46 (SE 0.13) for the natural logarithm of endotoxin (p = 0.0003), which means that each 1.0 unit increase in the natural logarithm of airborne endotoxin concentration was associated with 0.46 more illness episodes per year. An alternative expression of the relation would be that a doubling of air endotoxin concentration was associated with an increase of 0.32 illness episodes per year (p = 0.0003), adjusted for age, year of study, breast-feeding, environmental tobacco smoke, child care attendance, indoor temperature, and income. Also, starting from the geometric mean (0.49) and increasing endotoxin by its geometric mean resulted in 4.7% excess illnesses per year. Similarly, doubling air endotoxin was associated with an increase of 3.25 illness days per year (p = 0.005), adjusted for age, year of study, breast-feeding, child care attendance, indoor temperature, and sex. Starting from the geometric mean (0.49) and increasing endotoxin by its geometric mean resulted in 5.5% excess illness days per year.

Significant covariates in the regression of illness episodes were year of testing, indoor temperature, age, child care, environmental tobacco smoke, and income (all p < 0.05). Similar results were found with illness days with a β-coefficient of 4.68 (SE 1.66, p = 0.005).

In Figure 1, the β-coefficient for the effect of the natural logarithm of endotoxin on illness episodes and total illness days is graphed against the size of the window. The magnitude of the association between illness episodes and endotoxin levels was almost linearly decreasing.

Table 2. Characteristics of children's home environments overall and stratified by terciles of endotoxin.

| Variable | Overall | 1st tercile | 2nd tercile | 3rd tercile | p-Valuea |
|----------|---------|------------|------------|------------|----------|
| Children |         |            |            |            |          |
| Age (days) | 332 | 351 ± 42.1 | 355 ± 34.9 | 337 ± 59.0 | 360 ± 19.6 | 0.38 |
| Mean hours/week child care | 332 | 7.09 ± 8.97 | 8.43 ± 9.18 | 7.65 ± 8.48 | 7.60 ± 9.28 | 0.49 |
| Percent postnatal interviews where breast-feeding reported | 332 | 30.1 ± 26.7 | 31.0 ± 25.4 | 29.2 ± 23.5 | 29.9 ± 25.1 | 0.78 |
| Environment |         |            |            |            |          |
| Endotoxin (EU/m³) | 332 | 0.49b ± 3.49c | 0.14b ± 2.32c | 0.50b ± 1.32c | 1.80b ± 2.10c | — |
| Living room ergosterol (ng/m³) | 319 | 0.15b ± 3.94d | 0.16b ± 3.84d | 0.15b ± 3.30d | 0.14b ± 4.63d | 0.40d |
| Bedroom ergosterol (ng/m³) | 319 | 0.14b ± 4.24d | 0.14b ± 4.14d | 0.15b ± 3.30d | 0.14b ± 4.71d | 0.81d |
| Temperature (°C) | 332 | 20.9 ± 2.32 | 20.9 ± 2.24 | 20.7 ± 2.15 | 21.0 ± 2.33 | 0.97 |
| Relative humidity (%) | 332 | 31.5 ± 6.24 | 30.8 ± 5.74 | 31.5 ± 5.80 | 33.0 ± 6.52 | 0.01 |
| Interior wood storage (m³) | 332 | 51.3 ± 29.3 | 50.3 ± 29.1 | 51.4 ± 30.5 | 50.7 ± 28.2 | 0.93 |

*p-Value of the Fisher test for a linear trend for the terciles of endotoxin. | bGeometric mean. | cGeometric SD. | dGeometric mean. | eGeometric SD. | fGeometric mean. | gGeometric SD.

Table 3. Incidence of children’s illness overall and stratified by terciles of endotoxin concentrations.

| Variable | Overall | 1st tercile | 2nd tercile | 3rd tercile | p-Valuea |
|----------|---------|------------|------------|------------|----------|
| No. of episodes of any illness² per year | 332 | 6.83 ± 2.80 | 6.66 ± 2.86 | 6.60 ± 2.85 | 7.22 ± 2.85 | 0.13 |
| No. of illness days per year | 332 | 58.5 ± 36.4 | 54.6 ± 34.4 | 57.5 ± 36.4 | 63.5 ± 38.0 | 0.07 |
| No. of days with cough per year | 332 | 33.0 ± 25.3 | 31.9 ± 23.8 | 31.0 ± 24.4 | 36.1 ± 27.6 | 0.21 |
| No. of days with wheeze per year | 332 | 4.06 ± 9.66 | 2.28 ± 4.55 | 4.26 ± 10.1 | 5.66 ± 12.4 | 0.01 |
| No. of days with SOB per year | 332 | 0.41 ± 1.20 | 0.30 ± 0.99 | 0.48 ± 1.25 | 0.46 ± 1.33 | 0.30 |
| No. of days with stuffy nose per year | 332 | 48.7 ± 31.2 | 46.1 ± 29.7 | 48.1 ± 31.3 | 51.9 ± 32.7 | 0.17 |

²SOB, shortness of breath.
with the use of longer observation periods extending further from the original sampling. Because the effect of endotoxin levels on illness episodes was highly significant for a 2-year period, it would be even more significant for shorter observation periods.

**Discussion**

Air endotoxin was positively associated with an increase in episodes of respiratory illness among children during their first 2 years of life despite adjustment for many host and environmental factors, including indicators of fungal exposure. The method of endotoxin collection is unique compared with previous

**Table 4. Association between illness episodes (dependent variable) and natural logarithm of endotoxin concentrations: multiple linear regression analysis.**

| Independent variable     | β   | SE  | p-Value |
|--------------------------|-----|-----|---------|
| Intercept                | 6.99| 0.18| < 0.0001|
| Ln(endotoxin)            | 0.24| 0.12| 0.0556  |
| Adjusted (model $R^2 = 0.18$) |    |     |         |
| Intercept                | 7.74| 1.92| < 0.0001|
| Ln(endotoxin)            | 0.46| 0.13| 0.0003  |
| Year tested              |     |     |         |
| 1998                     | −0.77| 0.55| 0.1645  |
| 1999                     | −1.23| 0.55| 0.0269  |
| 2000                     | −0.37| 0.56| 0.5019  |
| 2001                     | 1.18 | 0.56| 0.3231  |
| 2002                     | −0.90| 0.52| 0.3881  |
| 2003 Reference           |     |     |         |
| Temperature              | −0.21| 0.06| 0.0012  |
| Age                      | 0.01 | 0.004| 0.0386  |
| Breast-feeding           | 0.94 | 0.58| 0.1031  |
| Child care               | 0.04 | 0.02 | 0.2000  |
| Exposure to smoke        |     |     |         |
| Low                      | −0.70| 0.39| 0.0757  |
| Medium                   | 0.17 | 0.37| 0.6358  |
| High Reference           |     |     |         |
| Income                   |     |     |         |
| <$30,000                 | −0.57| 0.41| 0.1614  |
| $30,000–49,999           | −0.93| 0.33| 0.0056  |
| ≥$50,000                 |     |     |         |
| Effect size              |     |     |         |
| Figure 1. The effect size over time between illness and bedroom endotoxin measured at birth. (A) Annualized illness episodes. (B) Annualized illness days. Effect size is represented as the β-coefficient for the effect of the natural logarithm of endotoxin on illness along with its 95% confidence interval. |  | | |

**Table 5. Association between illness days (dependent variable) and natural logarithm of endotoxin concentrations: multiple linear regression analysis.**

| Independent variable     | β   | SE  | p-Value |
|--------------------------|-----|-----|---------|
| Intercept                | 6.07| 2.28| < 0.0001|
| Ln(endotoxin)            | 3.09| 1.59| 0.0533  |
| Adjusted (model $R^2 = 0.15$) |    |     |         |
| Intercept                | 4.73| 2.47| 0.0565  |
| Ln(endotoxin)            | 4.68| 1.66| 0.0050  |
| Year tested              |     |     |         |
| 1998                     | −1.50| 6.90| 0.8279  |
| 1999                     | −4.88| 7.01| 0.4863  |
| 2000                     | 4.77 | 7.08| 0.5003  |
| 2001                     | 13.2 | 7.17| 0.0674  |
| 2002                     | −7.77| 6.78| 0.2528  |
| 2003 Reference           |     |     |         |
| Temperature              | −2.73| 0.85| 0.0014  |
| Age                      | 0.16 | 0.06 | 0.0029  |
| Breast-feeding           | 0.70 | 0.22 | 0.0013  |
| Child care               | 18.3 | 7.45| 0.0147  |
| Sex (male)               | 7.36 | 3.79| 0.0533  |
irrespective of parental history of atopy, using airborne rather than dust endotoxin, including indicators of respiratory illness in addition to wheeze, and considering confounding by indoor mold exposure. Nevertheless, even with children not selected based on atopic parents, we also found that wheeze was the symptom with the strongest association with endotoxin, and the effect size became smaller with increased duration of follow-up.

Sources of endotoxins. Gram-negative bacteria are found in water, soil, and outdoor air. Reported indoor sources of gram-negative include contaminated humidifiers, pets, storage of food waste, and increased amounts of settled dust (Park et al. 2001b). The need for water availability is consistent with our finding that relative humidity was positively associated with air endotoxin, not previously described. Gehring et al. (2001a) found that dust concentrations were higher with cats and dogs present. These studies found that endotoxin was higher in old buildings, with longer duration of occupancy, low ventilation rate, and poor housekeeping. Indoor pets were not associated with air endotoxin in the present study, which was carried out during the cold season with frozen ground and often snow cover. Perhaps pets would be less likely to go outside and subsequently bring in soil on their paws.

In summary, the present study supports a positive association between airborne endotoxins and the incidence of acute respiratory illnesses during the first 2 years of life, independent of allergic history and exposure to indoor mold that may coexist with contamination by bacterial endotoxin.

References

Douwes J, Pearce N, Heederik D. 2002. Does environment endotoxin exposure prevent asthma? Thorax 57:86–90.
Douwes J, Zooldhof A, Doekes G, van der Zee S, Wouters I, Boezen HM, et al. 2000. (1—>3)-β-D-Glucan and endotoxin in house dust and peak flow variability in children. Am J Respir Crit Care Med 162:1348–1354.
Foto M, Vrijmoed LLP, Miller JD, Ruest K, Lawton M, Dales RE. 2005. Comparison of airborne ergosterol, glucan and Air-O-Cell data in relation to physical assessments of mold damage and some other parameters. Indoor Air 15:257–266.
Gehring U, Bolte G, Borte M, Bischof W, Fahlbusch B, Wichmann HE, et al. 2001a. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. J Allergy Clin Immunol 108:157–166.
Gehring U, Bouwes J, Doekes G, Koch A, Bischof W, Fahlbusch B, et al. 2001b. (1,3)-β-D-Glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. Environ Health Perspect 109:139–144.
Lapa e Silva JR, Pessebon da Silva MD, LeFart J, Vargaftig BB. 2000. Endotoxins, asthma, and allergic immune responses. Toxicology 152:31–35.
Lianjua AA, Milton DK, Cedoric JC, Ryan L, Weiss ST, Gold DR. 2002. A longitudinal analysis of wheezing in young children: the independent effects of early life exposure to house dust endotoxin, allergens, and pets. J Allergy Clin Immunol 110:736–742.
Michel O, Ginanni R, Duchateau J, Vertongen F, Le Bon B, Serygael S. 1991. Domestic endotoxin exposure and clinical severity of asthma. Clin Exp Allergy 21:441–448.
Michel O, Kips J, Duchateau J, Vertongen F, Robert L, Collet H, et al. 1996. Severity of asthma is related to endotoxin in house dust. Am J Respir Crit Care Med 154:1641–1646.
Park J-H, Gold DR, Spiegelman DL, Burge HA, Milton DK. 2001a. House dust endotoxin and wheeze in the first year of life. Am J Respir Crit Care Med 163:322–328.
Park J-H, Spiegelman DL, Burge HA, Gold DR, Chev GL, Milton DK. 2000. Longitudinal study of dust and airborne endotoxin in the home. Environ Health Perspect 108:1023–1028.
Park J-H, Spiegelman DL, Gold DR, Burge HA, Milton DK. 2001b. Predictors of airborne endotoxins in the home. Environ Health Perspect 109:859–864.
Reed CE, Milton DK. 2001. Endotoxin-stimulated innate immunity: a contributing factor for asthma. J Allergy Clin Immunol 108:157–166.
Rizzo MC, Naspolini CK, Fernandez-Caldas E, Lackey RF, Minicas L, Sole D. 1997. Endotoxin exposure and symptoms in asthmatic children. Pediatr Allergy Immunol 8:121–126.
Rylander R. 2002. Endotoxin in the environment—exposure and effects. J Endotoxin Res 8:241–252.
Samet JM, Lambert WE, Skipper BJ, Cushing AH, McLaren LC, Schwab M, et al. 1992. A study of respiratory illnesses in infants and ND2 exposure. Arch Environ Health 47:63.
Verhoef AP, Burge HA. 2004. Health risk assessment of fungi in home environments. Ann Allergy Asthma Immunol 78(6):544–554.
Von Ehrenstroos OS, von Mutius E, Illi S, Bauman L, Bohm O, von Kries R. 2000. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 30:187–193.
Von Mutius E, Braun-Fahrlander C, Schierl R, Riedler J, Ehlenmann S, Maisch S, et al. 2000. Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clin Exp Allergy 30:1230–1234.