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Fungi and pollen exposure in the first months of life and risk of early childhood wheezing

Kim G. Harley, PhD,

School of Public Health, University of California, Berkeley, California, USA

Janet M. Macher, ScD, MPH,

California Department of Public Health, Richmond, California, USA

Michael Lipsett, MD,

California Department of Public Health, Richmond, California, USA

Paurene Duramad, PhD,

School of Public Health, University of California, Berkeley, California, USA

Nina T. Holland, PhD,

School of Public Health, University of California, Berkeley, California, USA

Steven S. Prager, MD,

Central Coast Allergy and Asthma, Salinas, California

Jeannette Ferber, MPH,

School of Public Health, University of California, Berkeley, California, USA

Asa Bradman, PhD,

School of Public Health, University of California, Berkeley, California, USA

Brenda Eskenazi, PhD, and

School of Public Health, University of California, Berkeley, California, USA

Ira B. Tager, MD

School of Public Health, University of California, Berkeley, California, USA

Abstract

BACKGROUND—Many studies have found that risk of childhood asthma varies by month of birth, but few have examined ambient aeroallergens as an explanatory factor.

OBJECTIVE—To examine whether birth during seasons of elevated ambient fungal spore or pollen concentrations is associated with risk of early wheezing or blood levels of Th1- and Th2-type cells at 24 months of age.

METHODS—514 children were enrolled before birth and followed to 24 months of age. Early wheezing was determined from medical records and Th1 and Th2-type cells were measured in peripheral blood using flow cytometry. Ambient aeroallergen concentrations were measured throughout the study period and discrete seasons of high spore and pollen concentrations were defined.

Corresponding Author: Kim Harley, PhD, Center for Children's Environmental Health Research, School of Public Health, U.C. Berkeley, 2150 Shattuck Ave, Suite 600, Berkeley, CA 94704, Tel: 510-643-1310, Fax: 510-642-9083, kharley@berkeley.edu.

COMPETING INTERESTS

None

RESULTS—A seasonal pattern was observed, with birth in the fall-winter (the fungal spore season) associated with increased odds of early wheezing (adjusted odds ratio = 3.1; 95% confidence interval: 1.3, 7.4). Increasing mean daily concentrations of basidiospores and ascospores in the first three months of life were associated with increased odds of wheeze, as were increasing mean daily concentrations of total and specific pollen types. Levels of Th1 cells at age 24 months were positively associated with mean fungal spore concentrations and negatively associated with mean pollen concentrations in the first three months of life.

CONCLUSIONS—Children with higher exposure to fungal spores and pollen in the first three months of life were at increased risk of early wheezing. This association was independent of other seasonal factors, including ambient PM_{2,5} levels and lower respiratory infections.

Keywords

asthma; wheeze; birth season; mold; pollen; epidemiology

INTRODUCTION

Multiple studies have found month of birth to be associated with the risk of allergic sensitization[1–4] or asthma[5–12] later in life, an observation suggesting that exposure to seasonal allergens in the perinatal period may contribute to the development of atopic disease. However, previous studies have shown little consistency as to which months are associated with the highest asthma risks and whether these months are associated with higher ambient concentrations of specific aeroallergens, such as fungi or pollen. Additionally, month of birth may represent exposures other than aeroallergens, including respiratory syncytial virus (RSV)[5], air pollution, and residential dampness, all of which have been associated with development of early childhood wheeze and asthma.[13]

Aeroallergen exposure is clearly associated with asthma exacerbations,[14, 15] but the role of such exposures in disease induction is less clear. The initial priming of T-cells to respond to allergens is thought to occur during late gestation and the neonatal period, and early-life exposure to ambient allergens may reinforce T-cell switching to favor a predominance of Th2 cells.[16] Kihlström *et al.*[17, 18] found that children born in the three months before a season of extremely high birch pollen were at increased risk of asthma at age five, but children born after this season (whose mothers were exposed during pregnancy) were not. A cohort study of children at risk for atopic disease found that higher concentrations of *Penicillium* spp. and total fungal spores in living room air of infants 2–4 months of age were associated with an increased risk of wheeze and persistent cough at age 12 months.[19, 20] Another longitudinal study reported that higher spore concentrations in living room air collected when infants were 2–3 months old were associated with increased risks of lower respiratory infection at one year of age[21] and of allergic rhinitis at age five.[22] Recent work also implicates early-life exposure to airborne particulate matter (PM) with development of asthma and allergen sensitivity in young children in the Netherlands.[23]

In the present analysis we examine whether the association between month of birth and early wheezing at age two could be explained by high ambient fungal spore or pollen exposure in the first three months of life. Additionally, we measured levels of Th1- and Th2-type cells in peripheral blood at age 24 months to determine whether cytokine profiles were associated with early life aeroallergen exposure.

METHODS

Participants and Recruitment

Subjects were children in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), a birth cohort study that is investigating the effects of environmental exposures on the health of low-income, predominantly Mexican immigrant families in an agricultural region of California. Pregnant women were recruited between October 1999 and October 2000 through collaborating prenatal clinics. Women were eligible to participate if they were less than 20 weeks' gestation, 18 years or older, spoke English or Spanish, qualified for low-income government health insurance (Medicaid), and planned to deliver at the county hospital. Of 1,130 eligible women, 601 agreed to participate, and 536 newborns were enrolled in the study. The present analysis was limited to 514 children with complete medical record data through 24 months of age. Blood samples for measurement of Th1 and Th2 cytokines were available for 236 of these children at the 24-month visit (median age: 23.9 months; IQR: 0.9 months; range: 21.7–29.0 months). Study procedures were approved by the University of California, Berkeley Committee for the Protection of Human Subjects.

Data Collection

Pediatric medical records were collected from all facilities where the child had received care between birth and 24 months of age. A single registered nurse abstracted relevant information onto standardized forms. A child was considered to have early wheezing if medical records indicated a clinician's diagnosis of asthma at any time between birth and 24 months of age. Because many of these children may not continue to have asthma at later ages[24], we considered this diagnosis to represent "early wheezing" rather than asthma.

Standardized interviews were administered to the mothers during pregnancy, after delivery, and when the children were approximately six months (median: 6.4 months; IQR: 1.0 month; range: 5.5–11.9 months) and 12 months (median: 12.4 months; IQR: 1.5 months; range: 11.1–22.8 months) of age. Interviews assessed demographic data and child exposures (e.g., duration of breastfeeding, second-hand smoke, frequency of colds, and presence of pets in the home), and were conducted in English or Spanish by bilingual, bicultural interviewers. Home inspections were conducted by trained inspectors when the children were 6 and 12 months of age to assess environmental exposures (e.g., wall moisture measurements, visible fungal growth, evidence of cockroach or rodent infestation, and presence of a gas stove).

Blood was analyzed for Th1- and Th2-associated cytokines if the sample was received at the study laboratory within 48 hours of collection. Methods for Th1 and Th2 analysis have been described previously.[25] Briefly, 500 μ l of whole blood were activated for four hours with phorbol 12-myristate 13-acetate (PMA) and ionomycin (Sigma-Aldrich, St. Louis, MO) to stimulate cytokine production. Fluorescent stain was then applied with antigen-specific antibodies for CD4+ T-lymphocytes (PerCP, Becton Dickinson, Franklin Lakes, NJ), IFN- γ (IFN- γ /FITC, Becton Dickinson), and IL-4 (IL-4/PE, Becton Dickinson). With flow cytometry, the CD4+ population was identified and gated; these cells were then examined for expression of IFN- γ and IL-4. Cells that stained positive for IFN- γ were classified as Th1 cells, and those that stained positive for IL-4 were classified as Th2 cells. Th1 and Th2 percentages were defined as the proportion of CD4+ cells identified as IFN- γ or IL-4 positive, respectively.

Measurement of ambient spore and pollen air concentrations

The study area (Salinas Valley, California) is a valley approximately 60 miles long and 10 miles wide, with a Mediterranean climate characterized by mild, rainy winters and dry summers. We measured fungal spore and pollen concentrations between October 1999 and July 2003, a period that encompassed the *in utero* period and first 24 months of life for all study children. The time frame of interest was exposure during the first three months of life.

Airborne spores and pollen were collected with a Hirst-type sampler[26] (Seven-Day Recording Volumetric Spore Trap; Burkard Manufacturing Co. Ltd., Rickmansworth, UK) located in the city of Salinas and placed 10 m above the ground to avoid over-representation of local vegetation. An AAAAI-certified analyst read three slides per week throughout the study period.

Twenty-seven spore and 48 pollen groups were identified. Seven fungal groups were observed on more than half of all sampling days and accounted for 82% of the annual spore concentrations: *Cladosporium* spp. (44%), basidiomycetes (25%), ascomycetes (6%), *Aspergillus* or *Penicillium* spp. (4%), *Botrytis* spp. (1%), smuts or myxomycetes (1%), and *Alternaria* spp. (0.5%). Ten plant groups were observed on more than 10% of all sampling days and accounted for 75% of the annual pollen concentrations: cypress (32%), oak (11%), unidentified pollen grains (8%), pine (7%), grass (6%), nettle/pellitory (4%), mulberry (4%), alder (4%), elm (3%), sage/wormwood (2%), and plantain (2%).

For each child, mean daily spore and pollen concentrations during the first three months of life were calculated for the four identified spore and seven identified pollen types that accounted for more than 3% of the total. The four spores and seven pollen also were summed to create variables for total spore and total pollen concentration in the first three months of life.

Measurement of PM concentrations

PM data were obtained from the Monterey Unified Air Pollution Control District (MBAPCD), which operated an air pollution monitoring station within ¼ mile of the study's aeroallergen station. For 24 hours every sixth day, the MBAPCD station measured PM with aerodynamic diameters 10 µm (PM₁₀) and 2.5 µm (PM_{2.5}) with high-volume Sierra-Andersen gravimetric samplers (Thermo Scientific, Waltham, MA). For each child, we calculated the mean 24-hour PM_{2.5} and PM₁₀ concentration during the first three months of life.

Seasonal Patterns of Exposure

To determine seasonal patterns of ambient exposures, we plotted daily concentrations of spores and pollen over the study period. A Lowess smooth[27] was fit to each scatter-plot, and high-concentration days were defined as those in which the fitted values were 40% of the maximum fitted value. High concentration days were clustered in time, resulting in three distinct periods each year: 1) high spore/low pollen concentration, termed the "spore season", 2) high pollen/low spore concentration, termed the "pollen season", and 3) periods with low concentrations of both. These seasons are shown in Figure 1, with the period of participants' births (February 16, 2000 through June 18, 2001) shown in black along the x-axis. Children were categorized according to season of birth, with low spore/low pollen as the reference category. There was very little overlap between the spore and pollen seasons; however, four children born between February 16 and March 1, 2000 overlapped the end of the spore season and the beginning of the pollen season. These children were classified as pollen season births because their primary early life exposure would be pollen. (Changing these children to spore season births did not change results markedly.)

We also examined seasonal patterns of PM air pollution using Lowess plots. Figure 1 shows that the periods of elevated PM_{2.5} tended to coincide with those of high spore concentrations. No seasonal patterns were observed for PM₁₀, but this is not surprising since the components of PM₁₀ (PM_{2.5} and PM_{10-2.5}) have different temporal peaks, which would tend to reduce PM₁₀ seasonality.

Data Analysis

We used logistic regression to examine the association between early wheezing and mean ambient spore and pollen concentrations in the first three months of life. Spore and pollen concentrations were negatively correlated and no children (including the 4 children born in the overlap of the spore and pollen seasons) simultaneously experienced high spore and pollen levels in the first three months of life; therefore, total spore and pollen exposure were not included in the same model. Separate models also were created for individual spore and pollen types. Because of the wide difference in ranges of spore and pollen concentrations, odds ratios were standardized to an increase in units equal to the interquartile range (IQR) for each exposure.

Associations between Th1 and Th2 profiles at age 24 months and mean spore and pollen concentrations were examined with linear regression. Th1, Th2, and the ratio of Th1:Th2 were log-transformed and three influential (outlier or leverage) points were excluded. Th1 and Th2 were analyzed both as absolute levels and as percentage of total CD4+ cells; as the results were similar by both approaches, we present only the latter.

To explore the possibility of confounding, we examined other early life exposures (Table 1) that we hypothesized might be associated with early wheezing. Variables of particular interest were: physician-diagnosed respiratory infections; visible mold, dampness, or evidence of cockroaches or rodents in the home; and PM_{2.5} exposure, all of which may display seasonal patterns. Exposures associated with both the exposure (season of birth) and outcomes (wheezing or Th1/Th2) were included as covariates in the multiple regression models. Covariates were kept in the model if they had a p-value <0.1 or if their exclusion changed the coefficient for the main effect by 10% or more. Because exposure to PM_{2.5} and fungal spores were highly correlated ($r = 0.6$), we regressed spore concentration on PM_{2.5} and used the residuals from this regression in the models that contained spore variables.

RESULTS

Table 1 shows the characteristics of the study population. Children were predominantly low income and of Mexican descent. Thirty-five children (6.8%) had a diagnosis of early wheezing by 24 months of age; a clear pattern was observed with month of birth (Figure 2). The unadjusted odds ratio for early wheezing for children born during the spore season relative to children born outside of the spore and pollen seasons was 2.8 (95% CI: 1.2, 6.5) (Table 1), an association that persisted after controlling for confounders (aOR: 3.1, 95% CI: 1.3, 7.4)(not shown). Poverty status, tobacco smoke exposure, and lower respiratory tract infection in the first year of life also were associated with increased odds of early wheezing, and higher PM_{2.5} exposure, pets, gas stove, or rodents in the home were of borderline statistical significance. Wall moisture measured at age six months was associated with reduced odds of early wheezing.

In adjusted analyses, there was only a weak association between total spore concentration in the first three months of life and early wheezing (Table 2). However, basidiospore and ascospore exposure each were associated with increased odds of wheezing (aOR per IQR of exposure: 2.1 and 2.8, respectively). Higher total pollen concentration in the first three months was associated with increased odds of early wheezing (aOR = 2.0; 95% CI: 1.1, 3.9).

Increasing concentrations of cypress, oak, pine, alder, and mulberry pollen in the first three months of life were also associated with greater odds of early wheezing, of which the associations for cypress, pine, and alder were statistically significant.

The median level of Th1-type cells in this population was 3.4% of total CD4+ lymphocytes (IQR: 3.07; range: 0.03 – 21.6). The median level of Th2-type cells was 0.9% of total CD4+ cells (IQR: 0.8; range: 0.4 – 4.1). Previously, we have shown that early wheezing was associated with higher Th2 levels in this population.[28]

The associations between Th1, Th2 and Th1:Th2 ratio and ambient spore and pollen concentrations in the first months of life are shown in Table 3, with beta coefficients representing the percent change in Th1, Th2 or Th:Th2 associated with a unit increase in spore or pollen concentration equal to the IQR. Th1:Th2 ratio was positively associated with number of colds, and negatively associated with early PM_{2.5} exposure and having a gas stove in the home (not shown). Thus, analyses controlled for these variables. Ambient fungal spore concentration in the first three months of life was positively associated with Th1 levels and Th1:Th2 ratio. Early life pollen concentration was negatively associated with Th1 levels at age 2.

DISCUSSION

We found that children born during periods of high ambient fungal spore concentration (February 16, 2000-March 1, 2000 or August 21, 2000-January 10, 2001) were at greatest risk of early wheezing by age 24 months. Although high concentration of *total* ambient spores in early life was not associated with wheezing, high concentration of basidiospores and ascospores in the first three months was. Total pollen concentration in the first three months of life also was associated with increased odds of wheezing, with individual associations seen with cypress, pine, and alder pollen. These associations were independent of lower respiratory illnesses and PM_{2.5} exposure in the first three months of life, both of which also were associated independently with increased odds of wheezing. Control for other seasonal factors, such as dampness, cockroaches, and rodents in the home did not alter these findings.

Our finding of increased odds of wheezing with elevated ambient concentrations of certain fungi is somewhat consistent with Gent et al.,[20] who found higher indoor concentrations of *Penicillium* spp. and total fungi at two to four months of age to be associated with greater risk of wheezing at age one. Indoor fungi were not measured in this study, but ambient and indoor concentrations have been shown to be correlated and the relationship is seasonally dependent [29]. Indoor concentrations of *Penicillium/Aspergillus* (which cannot be distinguished and are reported as one group) often are elevated in damp homes [30], which may explain why our ambient measures were not associated with early wheezing.

To our knowledge, this is the first study to examine early-life exposure to multiple outdoor fungal groups. Basidiospores are often the most abundant outdoor allergen-bearing particle and, along with ascospores, are released during rainfall or as humidity increases [31], a finding that is consistent with the crude association of early wheezing and being born in the spore season, which coincides with the rainy season in the study area (Table 1). Gregory and Hirst first suggested basidiospores as possible allergen sources [32], but far less is known about the allergenicity of ascospores [30]. *Cladosporium* spp., although present in very high concentrations in this region during the spore season, were not associated with wheezing.

Other studies also support our finding of an association between early wheezing and pollen exposure in the first months of life. Kihlström et al.,[17, 18] observed increased risk of

sensitization and allergic asthma among Swedish children exposed to unusually high levels of birch pollen in the first months of life. Although birch pollen was not detected in this region, a strong association was observed with alder pollen and early wheezing. Alder and birch are closely related and the major allergens from members of the birch family have been shown to cross-react [33].

Despite the observed associations of early wheezing and a number of antigen spores and pollens, our T-helper phenotype data do not offer any insight into the likelihood that the associations are related to atopic sensitization. The extent to which early exposure to antigens is important with respect to subsequent risks is not well established and undoubtedly related to a complex interplay of genetic predisposition, other environmental exposures, and the intensity and duration of exposure to specific allergens [34]. Given the developmental processes that govern T-helper maturation in children [35] and the fact that most of these children with early wheezing will not develop asthma later in childhood [24, 36], it is not surprising that a clear association between a T-helper phenotype and antigen exposure was not observed in this study.

We have assumed that the first three months of life represent the critical time period for exposure to ambient allergens, based on previous studies [34]. The sequential aeroallergen seasons dictate that children with high spore exposure between 0 and 3 months of age are likely to have high pollen exposure between 3 and 6 months of age. Thus, it is possible that the increased odds of wheezing associated with basidiospores and ascospores may actually represent an effect of exposure to pollen at slightly later ages. However, the seasonal pattern also offers the advantage of clearly differentiating children with high spore and pollen exposure because children born during the spore season have low pollen exposure and vice versa. No children were highly exposed to both spores and pollen in the first three months of life.

An additional possibility is that other factors with seasonal patterns, such as PM_{2.5} or RSV, are confounding the association of pollens and spores with wheezing. PM_{2.5} was associated with early wheezing in this population. However, analyses of pollen and spores controlled for this variable. We were not able to control for RSV in this analysis. Wu et al [5] recently showed that infants in Tennessee who were approximately 4 months of age at the peak of the RSV season were at highest risk of developing asthma by age 5, and that the seasonal birth pattern for asthma directly mirrored the pattern for RSV bronchiolitis. Like our study, Wu et al found the highest risk of asthma among children born in the fall. The authors attribute this increase in risk to RSV exposure rather than ambient allergen exposure, which was not measured. We do not have information on the seasonal pattern of RSV infection in this population. However, if the RSV peak in our study region occurs between December and February, as it did in the Tennessee study, RSV could be another possible explanatory factor for the birth season pattern of asthma risk observed.

Few studies have examined the roles of spore, pollen, and PM exposures in the early postnatal period in the development of childhood asthma or chronic wheezing. A strength of this study is that it linked date of birth to specific periods of elevated ambient allergens. We used outdoor measurements of a large number of fungal and plant groups reduced to epidemiologically relevant categories [33] and time-resolved to participant data. The number of children with early wheezing in this study was small (N=35), leading to imprecise estimation of the odds ratios. However, despite the small number of cases, we found several risk factors to be significantly associated with early wheezing. Unfortunately, atopy status as measured by skin prick testing was not available for these children; therefore we were unable to differentiate atopic from non-atopic wheezing. This is relevant, since a large

number of non-atopic children with wheezing will lose their symptoms in later childhood [36].

In conclusion, we found that birth during periods of elevated spore or pollen concentration was associated with wheezing at age two years in this largely Mexican immigrant population. Our T-helper lymphocyte data do not provide evidence that the association of early aeroallergen exposure with wheezing is related necessarily to allergic sensitization in this group. Despite our lack of more specific data on atopic sensitization to aeroallergens, these latter findings are consistent with the expectation that most of our subjects will lose their wheeze in later childhood as has been observed in populations of largely European origin [36].

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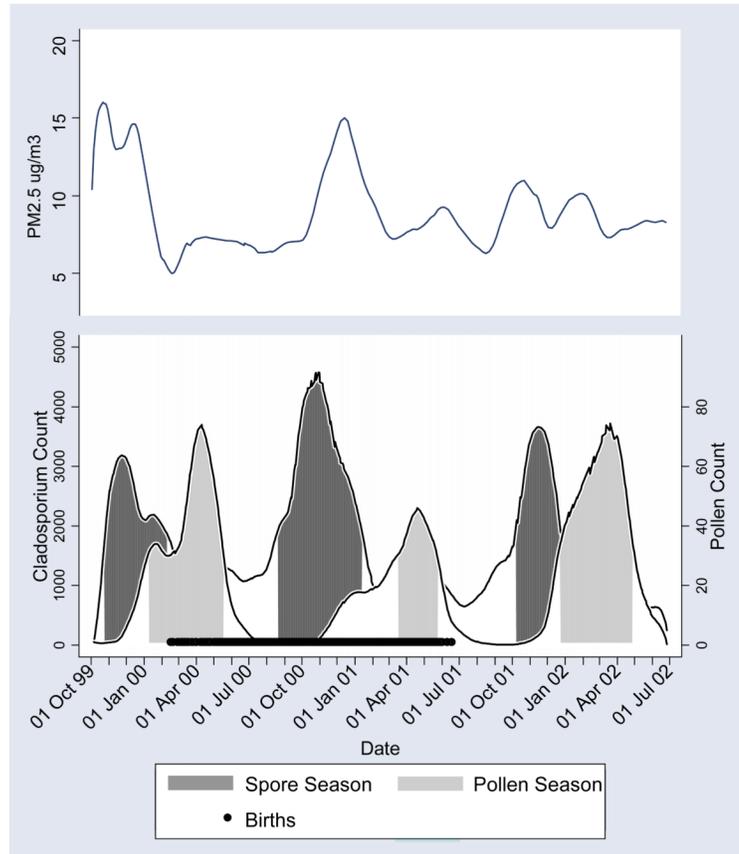


Figure 1. Lowess smoothing of daily spore and pollen concentration and mean PM_{2.5} levels. Salinas Valley, California, 1999–2002.

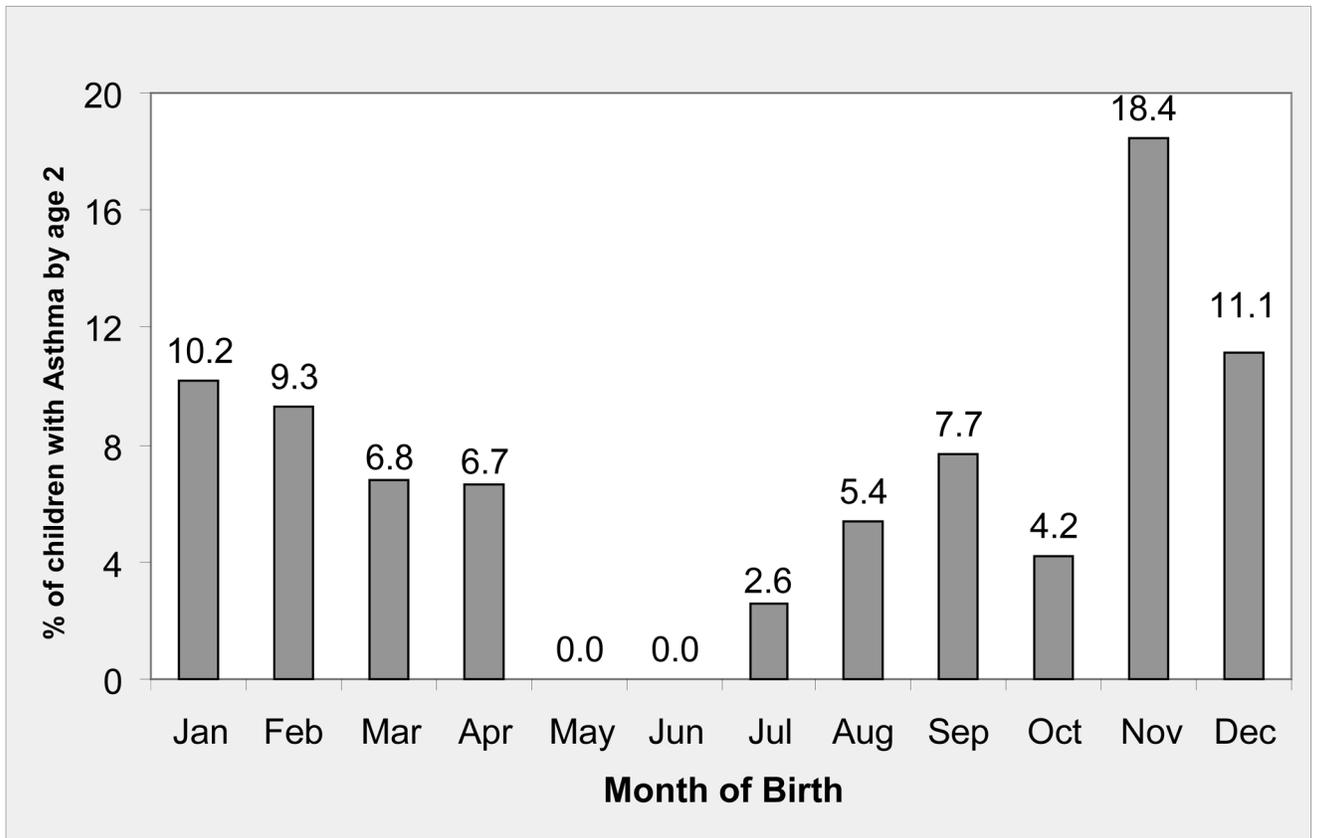


Figure 2.
Physician-diagnosed early wheezing, according to month of birth. Salinas Valley, California, 1999–2001.

Table 1

Selected characteristics of study population and associations with early wheezing (N = 514)

	N (%)	Early Wheezing
		Unadjusted OR (95% CI)
Baby's gender		
Boy	255 (49.6)	1.0
Girl	259 (50.4)	0.7 (0.4, 1.4)
Maternal Country of Birth		
United States	69 (13.5)	1.0
Mexico	433 (84.4)	0.6 (0.2, 1.4)
Other	11 (2.1)	0.9 (0.1, 8.0)
Family Income		
Above poverty level	171 (33.3)	1.0
At or below poverty level	343 (66.7)	2.5 (1.0, 6.2) *
Family History of Asthma		
No	417 (82.3)	1.0
Yes	90 (17.8)	1.4 (0.6, 3.2)
Breastfeeding Duration		
Never breastfed	28 (5.5)	1.0
6 months	272 (53.5)	1.2 (0.3, 5.4)
> 6 months	208 (40.9)	0.7 (0.1, 3.2)
Maternal Smoking during Pregnancy		
No	483 (94.2)	1.0
Yes	30 (5.9)	1.6 (0.5, 5.4)
Child Exposed to Tobacco Smoke (0–12 months)		
No	431 (92.1)	1.0
Yes	37 (7.9)	3.0 (1.2, 7.9) *
Signs of Cockroaches in home (0–12 months)		
No	140 (33.5)	1.0
Yes	278 (66.5)	0.9 (0.4, 1.9)
Signs of rodents in home (0–12 months)		
No	251 (60.1)	1.0
Yes	167 (40.0)	1.8 (0.9, 3.8)
Moderate-to-severe visible mold in home (0–12 months)		
No	148 (35.4)	1.0
Yes	270 (64.6)	0.8 (0.4, 1.7)
Any wall >17% moisture (6 months)		
No	273 (72.4)	1.0
Yes	104 (27.6)	0.3 (0.1, 1.0) *
Gas stove in home (0–12 months)		
No	91 (19.4)	1.0
Yes	377 (80.6)	3.8 (0.9, 16.4)

	Early Wheezing	
	N (%)	Unadjusted OR (95% CI)
Any pets (0–12 months)		
No	302 (67.0)	1.0
Yes	149 (33.0)	1.9 (0.9, 3.9)
Diagnosis of lower respiratory infection (0–12 months)¹		
No	350 (68.1)	1.0
Yes	164 (31.9)	4.0 (2.0, 8.2)*
Mean daily PM_{2.5} in the first three months of life		
< 8 µg/m ³	230 (44.8)	1.0
8 – 12 µg/m ³	218 (42.4)	1.7 (0.8, 3.7)
12 µg/m ³	66 (12.8)	2.4 (0.9, 6.4)
Season of birth		
Low spore/low pollen	203 (22.0)	1.0
Spore	195 (37.9)	2.8 (1.2, 6.5)*
Pollen	116 (22.6)	1.6 (0.6, 4.4)

¹ Diagnosis of bronchitis, bronchiolitis, pneumonia or croup noted in medical records

* p-value < 0.05

Table 2

Adjusted odds ratios for association of wheeze at age 2 years associated with mean daily ambient spore and pollen concentrations in first 3 months of life

	Median (Range)	Adjusted OR (95% CI)
Fungi¹		
<i>Cladosporium</i> spp. (OR per 1776 units) ³	1238 (610, 4180)	0.9 (0.5, 1.6)
Basidiospores (OR per 930 units)	550 (45, 1470)	2.1 (1.0, 4.4)*
Ascospores (OR per 203 units)	271 (82, 603)	2.8 (1.3, 5.9)*
<i>Aspergillus/Penicillium</i> spp.(OR per 145 units)	114 (25, 348)	0.8 (0.5, 1.5)
Total spores (OR per 2695 units)	2150 (1070, 6238)	1.2 (0.7, 2.0)
Pollens²		
Cypress (OR per 11 units)	4.4 (0, 29)	2.2 (1.2, 3.9)*
Oak (OR per 8 units)	0.5 (0, 16)	1.6 (0.9, 2.6)
Pine (OR per 4 units)	1.7 (0, 6)	2.8 (1.3, 6.0)*
Grass (OR per 4 units)	0.8 (0, 12)	1.0 (0.7, 1.6)
Alder (OR per 2 units)	1.0 (0, 4)	4.7 (1.3, 17.9)*
Elm (OR per 2 units)	0.2 (0, 6)	1.0 (0.7, 1.4)
Mulberry (OR per 2 units)	0 (0, 7)	1.6 (1.0, 2.6)
Nettle (OR per 1 units)	1.9 (1, 4)	0.9 (0.5, 1.6)
Total pollen (OR per 27 units)	18.6 (2, 61)	2.0 (1.1, 3.9)*

¹ Separate models for each fungal spore, controlling for gas stove in home, respiratory infection in first year of life, and PM_{2.5} in first three months of life (residuals independent of spores).

² Separate models for each pollen, controlling for gas stove in home, respiratory infection in first year of life, and PM_{2.5} in first three months of life.

³ Units equivalent to IQR of exposure for each spore or pollen type

* p-value < 0.05

Table 3

Adjusted regression of Th1, Th2, and Th1:Th2 ratio at age 24 months on total mean spore and pollen concentrations

	Th1	Th2	Th1:Th2
	Adjusted β^1 (95% CI)	Adjusted β^2 (95% CI)	Adjusted β^3 (95% CI)
Total spores (per 2695 units) ⁴	22.6 (1.9, 47.5)*	-11.7 (-25.4, 4.6)	38.8 (14.3, 68.6)*
Total pollen (per 27 units) ⁴	-20.3 (-33.5, -4.5)*	0.8 (-14.4, 18.6)	-13.5 (-28.7, 4.8)

¹Controlling for 2 colds in the first 6 months of life and PM_{2.5} in first three months of life.

²Controlling for presence of gas stove in home.

³Controlling for 2 colds in the first 6 months of life, PM_{2.5} in first three months of life, and gas stove in home.

⁴Equivalent to IQR of exposure

* p-value < 0.05