

Antibiotic exposure in early infancy and risk for childhood atopy

Christine Cole Johnson, PhD, MPH,^{a,b,c} Dennis R. Ownby, MD,^d Sharon Hensley Alford, MPH,^a Suzanne L. Havstad, MA,^a L. Keoki Williams, MD, MPH,^{c,e} Edward M. Zoratti, MD,^{b,f} Edward L. Peterson, PhD, MS,^{a,b} and Christine L. M. Joseph, PhD, MPH^{a,b}
Detroit, Mich, and Augusta, Ga

Background: The increase in pediatric allergy and asthma parallels the increase in use of antibiotics. Antibiotics disturb the flora of the gastrointestinal tract, possibly perturbing the developing immune system.

Objective: We evaluated whether antibiotic use during early infancy increased the risk for atopy.

Methods: Antibiotic prescriptions documented in medical records were collected from a birth cohort born from 1987 through 1989 (n = 725). At 6 to 7 years of age, 448 were followed by means of examination, including skin prick tests and serum IgE measurements to common allergens.

Results: Adjusted odds ratios (aORs) and 95% CIs were calculated comparing children with any versus those with no antibiotic use in the first 6 months and the outcomes of atopy (any positive skin test response), seroatopy (any positive specific IgE test result), either atopy or seroatopy, and both atopy and seroatopy. Atopy increased with antibiotic use approaching statistical significance (aOR, 1.48; 95% CI, 0.94-2.34; *P* = .09); however, the risk was concentrated among children with less than 2 pets in the home (aOR, 1.73; 95% CI, 1.07-2.80; *P* = .024) and children breast-fed for 4 or more months (aOR, 3.02; 95% CI, 1.27-7.17; *P* = .013). The aORs were generally in the same direction for seroatopy and the combined categories. **Conclusion:** Antibiotic use in early life appears to contribute to increased risk for atopy in certain subgroups of children. (*J Allergy Clin Immunol* 2005;115:1218-24.)

Key words: Allergy, antibiotics, atopy, children, IgE, skin testing

Sequential cross-sectional surveys in numerous locations suggest that the prevalence of pediatric allergy and asthma is increasing worldwide.¹ Strachan² proposed the hygiene hypothesis, which suggests that early exposure to

Abbreviation used

aOR: Adjusted odds ratio

infections decreases the risk for allergy and asthma. Holt et al³ proposed an immunologic model for the hygiene hypothesis, theorizing that early-life exposure to bacterial infections and bacterial products naturally present in the gut protects against atopy and asthma by favoring the development of a T_H1-predominant versus T_H2-predominant (allergic) cytokine profile in the maturing immune system.

Many lifestyle and medical care characteristics, including the use of antibiotics, have changed the patterns of infectious disease and bacterial exposure in infancy in the past 3 decades. Antibiotic use, in addition to the effect on the natural history of infection, is well known to alter gut flora.⁴ Although recent surveys in the United States suggest that antibiotic use among children might now be decreasing,⁵ the previous increase in antibiotic use among young children is coincident with the increasing prevalence of pediatric allergy and asthma.

The consideration of whether early antibiotic use is associated with increased risk for atopic disease has generated a surge of epidemiologic studies with conflicting results. From 1998 through 2002, a number of European studies examined whether antibiotic use increased the risks for allergy and asthma.⁶⁻¹³ These studies were cross-sectional,⁷⁻¹¹ relied on parental recall of antibiotic use,¹² or were based solely on record review.^{6,13} Although the studies included widely varying periods of antibiotic exposure and ages of children at measurement of disease outcome, all but one¹² suggested that antibiotic exposure increased the risk of pediatric atopy or asthma.^{6-11,13} Other studies have not supported this hypothesis.¹⁴⁻¹⁷

It has been demonstrated in allergy and asthma epidemiology that associations between a risk factor and an allergic outcome can be limited to particular subgroups of a population.¹⁸⁻²⁰ Generally, previous studies have considered the overall relationship between antibiotic use and atopy among all children combined, adjusting for, rather than stratifying by, other important risk factors. It is a well-known phenomenon that overall risk estimates implying no association can mask important relationships if other

From ^athe Department of Biostatistics and Research Epidemiology, ^cthe Center for Health Services Research, ^ethe Division of General Medicine, Department of Internal Medicine, and ^fthe Section of Allergy and Immunology, Division of Pulmonary, Critical Care, Allergy, Immunology and Sleep Medicine, Henry Ford Health System, Detroit; ^bEnvironmental Health Sciences Center in Molecular and Cellular Toxicology with Human Applications, Wayne State University, Detroit; and ^dthe Section of Allergy-Immunology, Medical College of Georgia, Augusta.

Supported by US National Institutes of Health (AI24156, AI50681, HL67427, PO3ES06639), the Blue Cross Blue Shield Foundation of Michigan, and the Fund for Henry Ford Hospital.

Received for publication August 25, 2004; revised April 14, 2005; accepted for publication April 19, 2005.

Reprint requests: Christine Cole Johnson, PhD, MPH, Henry Ford Health System, 1 Ford Place, 5C, Detroit, MI 48202. E-mail: cjohnso1@hfhs.org. 0091-6749/\$30.00

© 2005 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2005.04.020

factors are modifying the effect (interaction). The Detroit Childhood Allergy Study provides a large, prospectively followed, health maintenance organization–based birth cohort in which antibiotic prescriptions were documented by using comprehensive and complete unified group practice medical records, accompanied by data on numerous personal and environmental risk factors collected prospectively by personal interview. Atopic indicators were assessed by means of skin prick testing, serum IgE measurement, and a history and physical examination conducted by a pediatric allergist in a research setting when the cohort children were 6 to 7 years of age. We evaluated the influence of antibiotics during the first 6 months of life in relationship to these indicators of atopy and examined the effect of other important risk factors.

METHODS

Study population and follow-up

The recruitment of the Childhood Allergy Study population has been described elsewhere.²¹ All pregnant women enrolled in the medical group component of the largest Michigan health maintenance organization and living in a defined middle-class suburban area north of Detroit were potentially eligible. Women had to be 18 years of age or older, with a due date between April 15, 1987, and August 31, 1989, and were interviewed by study nurses during appointments in their obstetricians' offices after their first trimester. Children born at 36 weeks or later without the need for intensive care and a valid cord IgE measurement were continued in the study. The children were followed by means of telephone questionnaire at their first and third birthdays and by means of home visit at their second and fourth birthdays. At 6 to 7 years of age, the children underwent a clinical evaluation by a board-certified allergist (DRO). All aspects of the study were approved by the Henry Ford Hospital Human Rights Committee, and written informed consent was obtained from the subjects' families.

Exposure measurement–antibiotic use

Paper and electronic medical records of clinical encounters (ie, clinic visits, emergency department visits, and hospitalizations) were abstracted. Only children with a complete clinical record for at least the first 12 months, including evidence that medical group physicians served as the child's source of care, were included.

Information was collected regarding all prescribed antibiotics. Those prescribed within 7 days of each other were considered as a single course. Topical antibiotics and antifungal medications were excluded. Antibiotics were further grouped into those considered to have a relatively broad (penicillin combinations and second- and third-generation cephalosporins) versus narrow antibacterial spectrum.

Data were abstracted regarding respiratory illnesses, febrile episodes, and all conditions requiring antibiotics. Clinical encounters within 3 days of each other were considered part of the same event.

Outcomes measurement–atopic indicators

The clinical evaluation completed at age 6 to 7 years included a standardized history and physical, blood sample, and skin prick testing with the inhalant allergens *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, short ragweed, cat, dog, bluegrass, and *Alternaria* species (extracts from Bayer Biologics [now Hollister-

Stier], Spokane, Wash). Both positive (histamine, 1 mg/mL) and negative (glycerosaline) controls were used. Tests were applied by using the puncture method with a lancet (Bayer Biologics). Skin test responses were considered positive if the product of perpendicular wheal diameters was 4 mm or larger to any of the allergens tested and there was a flare of 10 mm or more when the negative control showed no reaction. (On the basis of the results of the positive and negative controls in the first 100 children, the value of 4 mm² was optimal for differentiating positive from negative results.) Atopy was defined as a positive skin test response to any of the 7 allergens tested. (*Alternaria* species was added after the study was in progress, and thus the first 44 children were not tested for this allergen. Of these 44, 34 results were negative to the other 6 allergens and were classified in the nonatopy category in our final analyses. The risk estimates were virtually the same if these children were excluded.)

Blood samples were measured for concentration of allergen-specific IgE antibodies by using a commercial assay (AlaSTAT; Diagnostic Products Corp, Los Angeles, Calif) for the same allergens used in skin testing, including *Alternaria* species. Specific IgE levels were expressed in international units per milliliter (1 IU/mL corresponds to 2.4 µg/L), and values of 0.35 IU/mL or greater were considered positive in accordance with the manufacturer's recommendation. Seroatopy was defined as any positive test result for allergen-specific IgE.

Statistical approach

χ^2 tests and logistic regression were used to calculate crude and adjusted odds ratios (aORs), as well as 95% CIs and *P* values, related to any versus no use of antibiotics in the first 6 months of life defined *a priori* and the following 4 outcome categories: atopy, seroatopy, atopy or seroatopy, and atopy and seroatopy. *P* values were considered statistically significant at the .05 level, and no adjustments were made to account for multiple comparisons. The aORs were adjusted for potential confounding variables defined before analyses, including child's sex, firstborn status, maternal history (mother reporting a history of asthma, hay fever, or allergies or allergen immunotherapy), breast-fed status (≥ 4 months of breast-feeding vs less), and pet exposure (2 or more cats and dogs in the home during the first year of life vs less), as well as fever (on the basis of findings from our previous work on this cohort^{22,23}) and reported day-care use and lower respiratory infections (croup, bronchitis, bronchiolitis, bronchospasm, influenza, lobe infiltrate, pneumonia, pneumonitis, and respiratory syncytial virus) in the first year of life. We evaluated whether there were any interactions between these potential confounders, antibiotics, and outcomes. Analyses were repeated considering only courses that included a broad-spectrum antibiotic versus no antibiotic to evaluate the *a priori* hypothesis that these drugs would alter gut flora to a greater extent. We also considered antibiotic exposure during the first 12 months of life versus later and first exposure at 0 to 3 months, 4 to 6 months, and 7 to 12 months, all versus after 12 months of age.

RESULTS

A total of 1194 pregnant woman were eligible, and 953 consented to their child's participation. The 835 children with valid cord blood IgE measurements were enrolled. Medical records were not retrievable for 51 children, and 59 children did not have at least 12 months of follow-up in the medical record (because of change of insurance, clinician, or residence), leaving 725 eligible children. Of these, 448 (61.8%) children underwent the clinical

TABLE I. Characteristics of children completing follow-up versus those who did not

Variable	Completed follow-up n/d (%)		P value
	Yes (n = 448)	No (n = 277)	
Female sex	227/448 (50.7)	146/277 (52.7)	.59
White*	420/448 (93.8)	251/277 (90.6)	.12
No. of older siblings			.73
0	173/391 (44.3)	114/250 (45.6)	
1	146/391 (37.3)	86/250 (34.4)	
≥2	72/391 (18.4)	50/250 (20.0)	
No. of dogs and cats in home in first year			.12
0	208/439 (47.4)	114/237 (48.1)	
1	162/439 (36.9)	73/237 (30.8)	
≥2	69/439 (15.7)	50/237 (21.1)	
Enrolled in day care in first year	178/406 (43.8)	96/235 (40.9)	.46
Breast-fed ≥4 mo	125/408 (30.6)	66/235 (28.1)	.49
≥1 parent with post-high school education	349/448 (77.9)	213/277 (76.9)	.75
One or both parents smoke	135/446 (30.3)	86/276 (31.2)	.80
Maternal history of allergy or asthma	171/447 (38.3)	109/277 (39.4)	.77
Diagnosis in first year of:			
Fever	207/436 (47.5)	129/277 (46.6)	.81
Croup-lower respiratory tract infection	128/448 (28.6)	72/277 (26.0)	.45
Use of antibiotics in the first 6 mo	221/448 (49.3)	150/277 (54.2)	.21

n/d, The numerator (n) represents the number of children with each variable and the denominator (d) the total number of children for whom we had information on that variable.

*On the basis of race-ethnicity of the mother.

examination at age 6 to 7 years, 176 children were unavailable, and 101 refused the examination. The baseline characteristics of children undergoing the examination were not significantly different from those not followed up for all evaluated variables, including the use of antibiotics (Table I).

There were 425 antibiotic prescriptions documented in the charts of 221 (49.3%) of the 448 children during the first 6 months of life. Of these prescriptions, 23 were noted within 7 days of another antibiotic and collapsed into one course, yielding 402 antibiotic courses. Of the 221 children prescribed antibiotics, 105 (47.5%) were given more than one course during this time period. The most common antibiotic category was the penicillins, with 135 children prescribed 1 course and 57 prescribed 2 or more courses, and 52 of the children received 1 or more courses of a broad-spectrum antibiotic.

Of the 443 children who underwent skin testing, 158 (35.7%) were classified as atopic. Of the 392 from whom blood was obtained, 149 (38%) were seroatopic. Combining these criteria, 45% were either atopic or seroatopic, and 36% were both atopic and seroatopic

(Table II). Table II displays the crude odds ratios and aORs for 1 or more versus no courses of antibiotics in the first 6 months. To increase statistical power and because the omission of these variables in the multivariable analyses did not materially change results, we did not adjust for sex, firstborn status, and day-care use in any of the final models. The aORs for atopy for all children reflected a modestly increased risk with antibiotic use that was of borderline statistical significance (aOR, 1.48; 95% CI, 0.94-2.34; $P = .09$).

We performed stratified analyses on the basis of pet keeping and breast-feeding because there was a statistical interaction between antibiotic use and these variables ($P = .01$ and $P = .03$, respectively) and with maternal history because of its well-known association with these outcomes. Among children with fewer than 2 pets in the home during the first 6 months of life, antibiotic use was associated with an increased risk for atopy (aOR, 1.73; 95% CI, 1.07-2.80; $P = .02$), whereas the opposite pattern was evident among children exposed to multiple pets (aOR, 0.25; 95% CI, 0.05-1.20; $P = .08$). For children breast-fed for 4 or more months, the aOR for antibiotic use and atopy was 3.02 (95% CI, 1.27-7.17; $P = .01$). Antibiotic use was not associated with an increased risk for atopic indicators in children breast-fed for less than 4 months. Risks were only slightly higher for those with a positive maternal history. We therefore evaluated children who had both fewer than 2 pets in the home and were breast-fed for more than 4 months. Antibiotic use was associated with an aOR for atopy of 3.98 (95% CI, 1.60-9.90; $P = .003$). The aORs were generally in the same direction but not usually statistically significant and of lesser magnitude for seroatopy and the categories combining atopy and seroatopy.

When we limited the comparisons to broad-spectrum antibiotics versus no use, the risks were similar to those for all antibiotics combined (data not shown). Considering the first 12 months of age versus later exposure for antibiotic exposure and atopy (Table III), the aORs were generally dampened, lost statistical significance, or both. Evaluating different ages of exposure, the most consistently high-risk estimates were associated with antibiotic use for the first time from 4 to 6 months, followed by 0 to 3 months. Antibiotic use was dramatically protective across each age of exposure period for children with multiple pets; however, this model could not adjust for fever because in this group the child had a fever in all but one case of antibiotic use. We also calculated aORs for antibiotic use in the first 6 months of life stratified by disease indication category (ear infection, eye infection, upper respiratory tract infection, and lower respiratory tract infection) and found no evidence that estimates varied by the diagnosis associated with antibiotic use (data not shown).

Children with exposure to less than 2 pets and a history of breast-feeding for 4 or more months were analyzed for a relationship between the number of antibiotic courses in the first 6 months (0, 1, 2, and 3+) with atopy and seroatopy. For atopy, the risk increased with increasing number of antibiotic courses (P for trend = .016). The

TABLE II. Use of antibiotics in the first 6 months of life versus later and atopy-seroatopy categories stratified by risk groups*

Risk groups (n positive/N total)	Any antibiotic use			
	OR (95% CI)	P value	aOR† (95% CI)	P value
Atopy				
All children (158/443)	1.22 (0.82-1.79)	.33	1.48 (0.94-2.34)	.09
Children with <2 pets (143/365)	1.53 (1.00-2.34)	.05	1.73 (1.07-2.80)	.02
Children with multiple pets (13/69)	0.51 (0.15-1.74)	.28	0.25 (0.05-1.20)	.08
Children breast-fed ≥4 mo (51/123)	2.10 (1.01-4.38)	.05	3.02 (1.27-7.17)	.01
Children breast-fed <4 mo (99/280)	0.96 (0.58-1.56)	.85	1.11 (0.64-1.92)	.72
Children with maternal history (60/168)	1.39 (0.74-2.61)	.31	1.91 (0.91-3.98)	.09
Children without maternal history (98/274)	1.11 (0.68-1.82)	.68	1.28 (0.71-2.31)	.41
Children with <2 pets and breast-fed (46/101)	3.51 (1.54-7.98)	.003	3.98 (1.60-9.90)	.003
Seroatopy				
All children (149/392)	0.98 (0.65-1.48)	.92	1.16 (0.72-1.87)	.54
Children with <2 pets (134/325)	1.14 (0.73-1.78)	.56	1.22 (0.74-2.01)	.43
Children with multiple pets (12/60)	0.77 (0.21-2.79)	.69	0.71 (0.15-3.30)	.66
Children breast-fed ≥4 mo (46/108)	1.73 (0.80-3.74)	.17	2.11 (0.88-5.02)	.09
Children breast-fed <4 mo (95/248)	0.78 (0.47-1.31)	.35	0.89 (0.50-1.59)	.70
Children with maternal history (55/140)	0.98 (0.50-1.94)	.96	1.34 (0.60-2.97)	.48
Children without maternal history (94/251)	0.97 (0.59-1.62)	.91	1.07 (0.58-1.96)	.84
Children with <2 pets and breast-fed (41/91)	2.09 (0.90-4.83)	.09	2.06 (0.83-5.07)	.12
Atopy or seroatopy				
All children (182/404)	1.10 (0.74-1.63)	.64	1.31 (0.82-2.09)	.25
Children with <2 pets (164/335)	1.31 (0.85-2.02)	.22	1.44 (0.88-2.35)	.14
Children with multiple pets (15/62)	0.77 (0.23-2.56)	.68	0.45 (0.10-2.12)	.31
Children breast-fed ≥4 mo (60/113)	2.10 (0.99-4.45)	.05	2.81 (1.17-6.72)	.02
Children breast-fed <4 mo (113/254)	0.82 (0.50-1.34)	.42	0.94 (0.54-1.66)	.84
Children with maternal history (70/148)	1.17 (0.61-2.23)	.64	1.56 (0.72-3.36)	.26
Children without maternal history (112/255)	1.05 (0.64-1.72)	.84	1.19 (0.65-2.17)	.57
Children with <2 pets and breast-fed (53/95)	3.05 (1.31-7.10)	.01	3.19 (1.28-7.96)	.01
Atopy and seroatopy				
All children (125/347)	1.02 (0.66-1.59)	.91	1.22 (0.74-2.03)	.44
Children with <2 pets (113/284)	1.26 (0.78-2.03)	.35	1.38 (0.81-2.35)	.24
Children with multiple pets (10/47)	0.51 (0.13-2.05)	.35	0.41 (0.07-2.25)	.30
Children breast-fed ≥4 mo (37/90)	1.91 (0.82-4.48)	.14	2.57 (0.97-6.83)	.06
Children breast-fed <4 mo (81/222)	0.81 (0.47-1.39)	.44	0.92 (0.50-1.68)	.78
Children with maternal history (45/123)	1.02 (0.49-2.13)	.96	1.43 (0.61-3.35)	.42
Children without maternal history (80/223)	1.01 (0.59-1.75)	.96	1.13 (0.59-2.17)	.70
Children with <2 pets and breast-fed (34/74)	2.86 (1.12-7.29)	.03	2.91 (1.07-7.93)	.04

*Atopy defined as 1 or more positive skin prick test response; seroatopy as 1 or more positive allergen-specific IgE test results.

†Adjusted for lower respiratory tract infections and fever in the first year of life and other variables on the table.

aOR for 1 course was 2.59 (95% CI, 0.88-7.63; $P = .085$), for 2 courses was 4.87 (95% CI, 0.79-29.9; $P = .088$), and for 3 or more courses was 5.15 (95% CI, 1.37-19.4; $P = .016$). However, a similar dose-response relationship was not evident for seroatopy or when considering all children.

DISCUSSION

The results from the Detroit prospective birth cohort suggest that antibiotic use is a risk factor for atopic indicators, specifically for children with other risk factors for atopy, as observed in 2 previous reports.^{6,11} A US birth cohort study of high-risk children in the Boston area did not implicate antibiotic use in the first year of life as a risk factor for the outcomes of asthma and atopy at age 5 years,

as reported by parents.¹⁴ However, odds ratios from this study were close to unity but had CIs that included estimates comparable with those for our overall group, and no data were presented stratifying by factors that were important in the Detroit cohort. Other studies investigating the hygiene hypothesis have determined that associations between exposures and outcomes are limited to certain risk groups. Data from the Tucson birth cohort have shown that extended breast-feeding was a risk factor for high IgE levels at the age of 6 and 11 years, but only among children born of mothers with high IgE levels themselves,¹⁸ and exposure to dogs was protective for frequent wheezing, but only for children without a positive family history of disease.¹⁹ We speculate that children with inherited propensity or lacking other protective exposures might be more susceptible to the effects of antibiotics. We hypothesize that children without exposure

TABLE III. Use of antibiotics and risk of atopy stratified by risk groups and age of first antibiotic exposure versus first exposure after 12 months of age

Risk groups (n)	Period of antibiotic use*	OR (95% CI)	P value	aOR (95% CI)†	P value
All children (443)	Any in 0-12 mo	1.19 (0.72-1.96)	.50	1.35 (0.74-2.47)	.32
	First use in 0-3 mo	0.96 (0.52-1.78)	.89	1.24 (0.60-2.60)	.56
	First use in 4-6 mo	1.58 (0.88-2.82)	.12	2.00 (0.99-4.05)	.05
	First use in 7-12 mo	1.07 (0.60-1.89)	.82	1.13 (0.59-2.17)	.71
Children with <2 pets (365)	Any in 0-12 mo	1.59 (0.93-2.72)	.09	1.80 (0.96-3.39)	.07
	First use in 0-3 mo	1.30 (0.67-2.53)	.43	1.72 (0.79-3.74)	.17
	First use in 4-6 mo	2.41 (1.28-4.55)	.007	2.82 (1.34-5.95)	.006
	First use in 7-12 mo	1.32 (0.72-2.42)	.37	1.45 (0.73-2.86)	.29
Children with multiple pets (69)	Any in 0-12 mo	0.13 (0.02-0.67)	.02	0.05 (0.01-0.41)‡	.005
	First use in 0-3 mo	0.09 (0.01-0.76)	.03	0.03 (0.01-0.46)‡	.01
	First use in 4-6 mo	0.16 (0.03-0.97)	.05	0.05 (0.01-0.58)‡	.02
	First use in 7-12 mo	0.11 (0.01-0.88)	.04	0.08 (0.01-0.77)‡	.03
Children breast-fed ≥4 mo (123)	Any in 0-12 mo	0.97 (0.39-2.61)	.95	1.18 (0.38-3.69)	.77
	First use in 0-3 mo	1.75 (0.52-5.84)	.36	2.78 (0.68-11.2)	.15
	First use in 4-6 mo	1.18 (0.39-3.57)	.77	1.82 (0.49-6.78)	.37
	First use in 7-12 mo	0.52 (0.17-1.64)	.27	0.63 (0.18-2.20)	.46
Children breast-fed <4 mo (280)	Any in 0-12 mo	1.16 (0.63-2.13)	.64	1.41 (0.69-2.89)	.35
	First use in 0-3 mo	0.70 (0.32-1.54)	.37	0.84 (0.34-2.09)	.72
	First use in 4-6 mo	1.55 (0.76-3.19)	.23	2.13 (0.91-5.01)	.08
	First use in 7-12 mo	1.25 (0.62-2.52)	.52	1.39 (0.64-3.02)	.40
Children with maternal history (168)	Any in 0-12 mo	1.25 (0.56-2.79)	.59	1.43 (0.57-3.56)	.44
	First use in 0-3 mo	0.79 (0.28-2.19)	.65	1.16 (0.36-3.69)	.80
	First use in 4-6 mo	1.69 (0.70-4.09)	.24	2.82 (0.95-8.38)	.06
	First use in 7-12 mo	0.86 (0.35-2.09)	.73	1.03 (0.37-2.88)	.96
Children without maternal history (274)	Any in 0-12 mo	1.28 (0.65-2.49)	.47	1.09 (0.51-2.33)	.83
	First use in 0-3 mo	1.08 (0.49-2.38)	.86	1.20 (0.45-3.17)	.72
	First use in 4-6 mo	1.52 (0.70-3.29)	.29	1.59 (0.63-4.06)	.33
	First use in 7-12 mo	1.26 (0.59-2.66)	.55	1.12 (0.48-2.63)	.79
Children with <2 pets and breast-fed (101)	Any in 0-12 mo	1.39 (0.49-3.94)	.53	1.55 (0.48-5.04)	.54
	First use in 0-3 mo	3.62 (0.95-14.1)	.06	5.08 (1.10-23.5)	.04
	First use in 4-6 mo	1.96 (0.58-6.62)	.27	2.47 (0.63-9.79)	.20
	First use in 7-12 mo	0.60 (0.18-2.00)	.41	0.76 (0.21-2.79)	.68

*With antibiotics first used at greater than 12 months used as the reference group.

†Odds ratios adjusted for lower respiratory tract infections and fever in the first year of life, as well as other variables on the table, including pet keeping; maternal history of asthma, hay fever, allergies, or allergen immunotherapy; and breast-feeding.

‡Odds ratios could not be adjusted for fever in the first year of life because of collinearity.

to the protection afforded by multiple pets²² might be more vulnerable to these effects. Children prescribed antibiotics in early infancy and who are breast-fed longer (or are given formula later) might be at somewhat higher risk for atopy.

In considering risk factors for pediatric atopic conditions, the timing of exposures might be particularly important. For example, Ball et al,²⁴ in the Tucson Children's Respiratory Study, reported that the first 6 months of life is the critical period when day-care use is protective for asthma. Considering antibiotic exposure, 2 previous reports^{7,10} considering age at initial antibiotic use suggested that it is very early exposure that confers the highest risk, although the majority of the previous studies considered only one exposure period, such as the first 1, 2, or 3 years of life. Oyama et al²⁵ have shown, in a mouse model, that both use of antibiotics during early life and timing of use have an effect on immunity. Administration of kanamycin in 3-week-old, but not 52-week-old, mice increased serum levels of total IgG1 and IgE, increased

in vitro IL-4 secretion, and reduced *in vitro* IFN- γ secretion, all markers suggestive of a T_H2 response. Our data suggest that exposure to antibiotics between 4 and 6 months, followed by between 0 and 3 months, confers the highest risk.

Recently reported studies using a murine model to demonstrate a distant immunologic effect on the lungs caused by a perturbation of the gut microbiota provide a plausible mechanism for the hypothesized antibiotic-atopy relationship.²⁶ In these experiments previously unsensitized mice given a 5-day course of oral antibiotics and subsequently a single dose of *Candida albicans* had a pulmonary response to an intranasal allergen (conidia of the mold *Aspergillus fumigatus* exposed 2 and 9 days after antibiotic use) exposure, which was typical of allergic sensitivity, including goblet cell metaplasia, and statistically significantly increased numbers of lung eosinophils and mast cells associated with dramatically increased production of serum IgE, IL-5, and IL-13. The animals showed no disease symptoms, and there was no evidence

of infection by *C albicans* in the lungs or other organs. These reactions were not observed in CD4 T cell–depleted mice, implying that the antibiotic and *C albicans* exposure altered a CD4 T_H2 response in the lungs. Moreover, they were able to repeat these findings when using other mouse strains and a nonfungal antigen, ovalbumin, as a challenge.²⁶

One potential confounding factor noted by others is that early lower respiratory tract infections, which are commonly treated with antibiotics, might be an early harbinger of atopy, referred to by some as a “reverse causation” bias. Adjusting our risk estimates by the occurrence of lower respiratory tract infections in the first 12 months of life mitigates our concern that this bias is driving our results. Furthermore, antibiotic use in the first 12 months combined and for the first time from 7 to 12 months was not associated with increased risk for the outcomes under study, which is contrary to expectations if this bias was systematic.

Another possible confounder is a child’s propensity to visit the doctor, increasing the likelihood of both antibiotic prescriptions and a doctor’s diagnosis of atopic disease.^{13,16} However, a variable can confound an association only if it is related to both exposure and outcome, and in our study all children were invited to the research-driven examination at which atopy and seroatopy were determined. Other possible confounders are maternal history, pet keeping, and breast-feeding. Women with a positive maternal history were not less likely to have multiple pets ($P = .35$) but were more likely to breast-feed for 4 months or longer ($P = .012$), but we adjusted for all of these variables when considering a particular risk factor in addition to other variables.

Although many of our results were statistically significant, many were imprecise because of sample size limitations and can only be considered suggestive. We were unable to distinguish between antibiotic use and fever, which has been shown to be protective in this cohort,²³ among children with multiple pets because of the relatively small sample size and the tight correlation between these variables that is characteristic of this specific group. Our results suggest that children exposed to antibiotics and multiple pets and fevers during the first year of life might be at remarkably low risk for atopy in midchildhood, regardless of timing of antibiotic use.

It is possible that even though we used visit history to determine that the children in these analyses were receiving their health care from medical group physicians, some children might have received an antibiotic from another physician and been unrecorded. We also made the assumption that children prescribed antibiotics actually ingested the medications. A substantial number of children were not followed up with clinical examination, although there were no differences in known baseline characteristics of this group. Finally, these results might only be generalizable to a Midwestern, white, middle-class population.

Complementary to the hygiene hypothesis, our data support the premise that antibiotic use in early life exerts

an important effect on the immune system and thereby increases the risk of atopic conditions in childhood in some children. We emphasize that we are not advocating that physicians not treat children with antibiotics in the first 6 months of life because these drugs are a critical part of the clinician’s armamentarium. However, these data bolster the current campaign within the public health and medical communities to avoid the inappropriate use of these vital drugs.²⁷

We acknowledge the work of the research staff who made this study possible, including the study nurses, laboratory coordinator and staff, data managers and programmers, and abstractors and allergy fellows who collected data from the medical records.

REFERENCES

1. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000;105(suppl):S466-72.
2. Strachan D. Family size, infection and atopy: the first decade of the “hygiene hypothesis”. *Thorax* 2000;55(suppl 1):S2-10.
3. Holt PG, Sly PD, Björkstén B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997;8: 53-8.
4. Levy J. The effects of antibiotic use on gastrointestinal function. *Am J Gastroenterol* 2000;95(suppl):S8-10.
5. Finkelstein JA, Stille C, Nordin J, Davis R, Raebel MA, Roblin D, et al. Decreasing antibiotic use among US children: the impact of changing diagnosis patterns. *Pediatrics* 2003;112:620-7.
6. Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. *Thorax* 1998;53:927-32.
7. Wickens K, Pearce N, Crane J, Beasley R. Antibiotic use in early childhood and the development of asthma. *Clin Exp Allergy* 1999;29: 766-71.
8. Alm JS, Swartz J, Lija G, Scheynius A, Pershagen G. Atopy in children of families with an anthroposophic lifestyle. *Lancet* 1999;353:1485-8.
9. Von Mutius E, Illi S, Hirsch T, Leupold W, Keil U, Weiland SK. Frequency of infections and risk of asthma, atopy and airway hyper-responsiveness in children. *Eur Respir J* 1999;14:4-11.
10. Wjst M, Hoelscher B, Frye C, Wichmann HE, Dold S, Heinrich J. Early antibiotic treatment and later asthma. *Eur J Med Res* 2001;6:263-71.
11. Droste JHJ, Wieringa MH, Weyler JJ, Nelen VJ, Vermeire PA, Van Bever HP. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? *Clin Exp Allergy* 2000;30: 1547-53.
12. Illi S, Von Mutius E, Lau S, Bergmann R, Niggemann B, Sommerfeld C, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study. *BMJ* 2001;322:390-5.
13. McKeever TM, Lewis SA, Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol* 2002;109:43-50.
14. Celedon JC, Litonjua AA, Ryan L, Weiss ST, Gold DR. Lack of association between antibiotic use in the first year of life and asthma, allergic rhinitis, or eczema at age 5 years. *Am J Respir Crit Care Med* 2002;166:72-5.
15. Cullinan P, Harris J, Mills P, Moffat S, White C, Figg J, et al. Early prescriptions of antibiotics and the risk of allergic disease in adults: a cohort study. *Thorax* 2004;59:11-5.
16. Bremner SA, Carey IM, DeWilde S, Richards N, Maier WC, Hilton SR, et al. Early-life exposure to antibacterials and the subsequent development of hay fever in childhood in the UK: case-control studies using the General Practice Research Database and the Doctors’ Independent Network. *Clin Exp Allergy* 2003;33:1518-25.
17. Foliaki S, Nielsen SK, Björkstén B, Von Mutius E, Cheng S, Pearce N. Antibiotic sales and the prevalence of symptoms of asthma, rhinitis, and eczema: the International Study of Asthma and Allergies in Childhood (ISAAC). *Int J Epidemiol* 2004;33:558-63.

18. Wright A, Sherrill D, Holberg CJ, Halonen M, Martinez FD. Breast-feeding, maternal IgE, and total serum IgE in childhood. *J Allergy Clin Immunol* 1999;104:589-94.
19. Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not atopy. *J Allergy Clin Immunol* 2001;108:509-15.
20. Johnson CC, Ownby DR, Havstad SL, Peterson EL. Family history, dust mite exposure in early childhood, and risk for pediatric atopy and asthma. *J Allergy Clin Immunol* 2004;114:105-10.
21. Ownby DR, Johnson CC, Peterson EL. Maternal smoking does not influence cord serum IgE or IgD concentrations. *J Allergy Clin Immunol* 1991;88:555-60.
22. Ownby DR, Johnson CC, Peterson EL. Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age. *JAMA* 2002;288:963-72.
23. Williams LK, Peterson EL, Ownby DR, Johnson CC. The relationship between early fever and allergic sensitization at age 6 to 7 years. *J Allergy Clin Immunol* 2004;113:291-6.
24. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538-43.
25. Oyama N, Sudo N, Kubo C. Antibiotic use during infancy promotes a shift in Th1/Th2 balance toward Th2-dominant immunity in mice. *J Allergy Clin Immunol* 2001;107:153-9.
26. Noverr MC, Noggle RM, Toews GB, Huffnagle GB. Role of antibiotics and fungal microbiota in driving pulmonary allergic responses. *Infect Immun* 2004;72:4996-5003.
27. Dowell SF, Marcy SM, Phillips W, Gerber M, Schwartz B. Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections. *Pediatrics* 1998;101:163-5.