

Effect of animal contact and microbial exposures on the prevalence of atopy and asthma in urban vs rural children in India

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Background: Environmental factors, including microbial exposures and close animal contact, are implicated in the lower prevalence of asthma and allergy in rural vs urban children.

Objectives: To determine (1) the prevalence of asthma, rhinitis, eczema, and atopic sensitization in rural and urban children in India; (2) differences in microbial and animal exposures in these locales; and (3) whether differences in environmental exposures account for the different rates of asthma and atopy in these locales.

Methods: One child from each of 50 urban (Mysore) and 50 rural (Vinobha) households in southern India was randomly selected for data analysis. Allergy, asthma, health, environment, and lifestyle information was obtained using a questionnaire and household inspections. Atopy was determined via skin prick testing for common allergens. Endotoxin content was measured in house dust samples.

Results: Children from rural vs urban areas had lower prevalences of self-reported asthma (8% vs 30%; $P = .005$), rhinitis (22% vs 42%; $P = .03$), and atopic sensitization (36% vs 58%; $P = .03$). Higher median dust endotoxin loads were found in rural vs urban households (6.50×10^4 EU/m² vs 1.27×10^4 EU/m²; $P < .001$). In multivariate analysis, close indoor animal contact (adjusted odds ratio [OR] 0.2; 90% confidence interval [CI], 0.05–0.9), outdoor animal contact (OR, 0.3; 90% CI, 0.1–0.8), and exclusive breastfeeding for at least 6 months (OR, 0.2; 90% CI, 0.1–0.5) were associated with lower atopic sensitization; mud flooring was associated with lower self-reported wheezing (OR, 0.1; 90% CI, 0.02–1.0).

Conclusion: Children in India who live with close animal contact and mud flooring and who were exclusively breastfed in infancy are less likely to develop asthma, rhinitis, and atopic sensitization.

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INTRODUCTION

In developing nations, rural locales seem to have lower rates of childhood asthma than urban locales.^{1–5} Animal contact and microbial exposures are some of the lifestyle and environmental factors that have been associated with less asthma.⁶ In India, where traditional lifestyles include close animal contact and associated microbial exposures, the trend of less asthma in rural locales has been reported recently.^{7–9} However, the relationship of animal and microbial exposures with asthma in Indian children has not been studied, to our knowledge. The relationship of such lifestyle features with rhinoconjunctivitis and allergen sensitization also has not been reported, to our knowledge.

We conducted the present study (1) to determine the prevalences of asthma, rhinitis, eczema, and atopic sensitization in rural and urban children in India; (2) to identify differences in microbial and animal exposures in rural vs urban locales; and

(3) to determine whether differences in environmental exposures account for the different rates of asthma and atopy in rural vs urban areas.

MATERIALS AND METHODS

The study was conducted in 2 locales in southern India: (1) the urban locale was the city of Mysore, with approximately 250,000 households and a population of 1 million, academic educational centers, and light industry, and (2) the rural locale was the village of Vinobha, 30 km to the west of Mysore, with 150 households and a population of approximately 600, with farming and stone cutting as the main occupations.

Study Population

Households in these 2 locales that had at least 1 child aged 6 to 16 years were eligible for inclusion in the study. Urban households were selected from 2 source populations representative of the middle class of the urban Mysore population: (1) students aged 6 to 16 years at a local music school and (2) residents of the living quarters of the Government Press. Rural households meeting the selection criteria were identified with the assistance of the village headman. A total of 103 households were selected for inclusion in the study (53 rural and 50 urban). A total of 164 children (97 rural and 67 urban) were enrolled. To eliminate potential confounding due to same-household linkages, 1 child from each household was

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randomly selected for the final data analysis. An ethical review board consisting of medical and nonmedical adults from the communities studied was convened to review the study protocol, methods, and ethical considerations of this study. Approval from this review board was granted. Written informed consent was obtained from the parents of every child.

Questionnaire and Inspection of Homes

The International Study of Asthma and Allergies in Children questionnaire¹ was translated into the local regional language of Kannada and was also modified to obtain more information regarding potentially relevant lifestyle and environmental issues, such as the domestic use of cow dung and indoor and outdoor contact with domesticated animals. The parents of all the children were interviewed by 2 of us (P.A.M. and A.D.H.). The interviews were conducted in private areas in the participants' homes, the village school, and the local music school in the city. Each household was also inspected, paying close attention to the proximity of outdoor and indoor domestic animals. Large animals, such as cattle, dogs, and cats, and smaller animals, such as poultry, mice, and rats, were taken into account. In addition, we noted the type of sleeping materials used and the type of fuel used in the kitchen area. Additional inquiries were made regarding the use of cow dung for regular domestic purposes, such as fuel use and as an antiseptic for cleaning and on the flooring, vs only for ceremonial religious purposes.

Skin Prick Testing

All the participating children underwent skin prick testing (SPT), using a sterile small pox needle, to a panel of perennial allergen extracts (Hollister Stier, Spokane, WA): house dust mite mix (a mixture of *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, 5,000 AU each), cockroach, cat, dog, and cattle. Histamine phosphate and human serum albumin or saline were used as positive and negative controls, respectively. The SPTs were performed on the volar aspect of the forearm by one of us (P.K.V.), who was certified by proficiency testing to perform SPTs for this study. This investigator was not involved in the interview process, to avoid information bias. The SPTs were read after 15 minutes. Wheal and erythema margins were measured in millimeters, recorded, and tape transferred. A positive SPT reaction was defined as a wheal 3 mm or greater in diameter compared with the negative control.

House Dust Collection and Endotoxin Load Determination

When permission was granted, dust samples were collected from (1) the floor of the room where the child or children slept and (2) the floor of the main living area of the family. At least 2 m² of the floor from each of the rooms was used for dust sampling, and both samples from each household were combined in a single sterile plastic bag with a wire closure (Fisher Scientific International, Hampton, NH). In several rural homes, the sleeping area and the living area were the same. For each sampled household, we aimed to collect at

least 1 tablespoon of fine dust. If greater than 2 m² was used for collection, such information was used to calculate the grams of dust collected per square meter of space for each household. Fine dust was collected using a new clean broom or brush for each home to avoid cross-contamination. Large pieces of debris and hair were excluded. After the dust was collected, the sample bags were appropriately labeled, stored in a freezer at -20°C, and batch-shipped to the National Jewish Medical and Research Center, where they were sieved and stored as dry dust in a -20°C nondefrosting freezer. For endotoxin quantitation, dust samples were sieved, suspended, sonicated, and assayed for endotoxin in biologically active endotoxin units using the *Limulus* amoebocyte lysate assay (QCL-1000; Bio-Whittaker, Walkersville, MD) under pyrogen-free conditions, as previously described.¹⁰ We calculated and compared the load of endotoxin collected per square meter from a limited set of 24 rural and 17 urban households.

Statistical Analysis

Quantitative data analysis was performed using a statistical software program (SPSS 9.0; SPSS Inc, Chicago, IL). Frequencies, univariate cross-tabulations, and multiple logistic regression were performed. Significance was defined at $P = .05$ for the univariate analyses. For the multivariate analysis, we used $P = .10$ to define significance to detect trends in the small study cohort.

RESULTS

A total of 164 children were enrolled in the study. To eliminate potential confounding due to same-household linkages, 103 children (1 from each household, randomly selected) were considered for the final analyses. Complete interview and SPT data were available on 100 of these children (50 urban and 50 rural). These 100 children formed the basis for the statistical results and conclusions described herein.

Table 1 provides the demographic characteristics of the urban and rural study participants. The age range of the children was 6 to 16 years, with a mean age of 10 years.

Table 1. Demographic Profile of Rural vs Urban Study Children in India

	Rural group (n = 50)	Urban group (n = 50)	P value
Age, mean, y	10.3	10.3	.97
Sex, female, %	46	36	.31
Siblings, mean, No.	2.3	0.8	<.001
Exclusively breastfed >6 mo, %	74	20	<.001
Went to preschool, %	14	88	<.001
Mud floor ever, %	66	4	<.001
Current smoker in house, %	66	14	<.001
Hepatitis B vaccination, %	10	54	<.001
Measles vaccination, %	100	100	NS
DPT vaccination, %	100	100	NS
Oral poliomyelitis vaccination, %	100	100	NS

Abbreviations: DPT, diphtheria, pertussis, tetanus; NS, not significant.

Although the age and sex composition did not differ between the 2 groups, other factors were notably different. Specifically, rural children had more siblings, were more likely to have been exclusively breastfed until at least 6 months of age, were more likely to have lived on a mud floor, were more likely to have been raised at home in early childhood, were more likely to be exposed to passive tobacco smoke indoors, and were less likely to have attended preschool. The proportions of urban and rural children who received vaccinations against measles, pertussis, diphtheria, tetanus, and poliomyelitis did not significantly differ, but rural children were less likely to have had a hepatitis B vaccination.

Exposure to animals was much greater for children in rural vs urban homes (Table 2). This increased exposure included small household animals, such as dogs, cats, and birds, and large farm-type animals, such as cattle, sheep, goats, and oxen. Indoor and outdoor animal exposure was greater in rural areas. Cow dung use exhibited an interesting pattern. Whereas regular daily dung use was far more common in the rural areas, occasional ceremonial dung use (for religious festivals or functions) was more common in the urban locale. In the subset of homes with available dust samples, the median endotoxin load in the urban homes (1.27×10^4 EU/m²; range, 0.04 – 10.09×10^4 EU/m²) was significantly lower than that in the rural households (6.50×10^4 EU/m²; range, 0.81 – 42.57×10^4 EU/m²; $P < .001$, Wilcoxon rank sum test) (Fig 1).

Table 3 provides a rural vs urban comparison of self-reported atopic diseases and SPT sensitivity results. The lifetime prevalence of self-reported rhinitis and wheezing was significantly lower in rural respondents. A similar pattern of lower rural prevalence was also seen with other allergy- and asthma-related items, such as “rhinitis in the past 12 months,”

“wheeze in the past 12 months,” and “exercise-induced bronchospasm” (data not shown). Atopy, defined as at least 1 positive SPT result, was also significantly less prevalent in rural children ($P = .03$). Skin test sensitivity was significantly lower in rural children for house dust mite and cockroach. However, although eczema was rarely reported, it was reported more often in rural children. Among reported asthmatic patients, 67% of urban children were atopic compared with 50% of rural children, although this difference was not statistically significant ($P = .61$).

We further investigated the specific environmental factors associated with wheezing and atopy, first by univariate analysis (Table 4). Rural locale, cow dung use (regular domestic vs ceremonial), indoor animal exposure, and exclusive breastfeeding were predictive of lower risk of wheezing and atopy. Mud flooring and siblings were correlated with lower risk of wheezing, but not significantly so for atopy. In addition, outdoor animal exposure and passive exposure to tobacco smoke inside the house were significantly associated with lower detected atopy, and nearly so for wheezing. Preschool attendance was associated with a higher risk of wheezing and atopy.

Table 5 gives the results of the multivariate analysis for predictors of atopy and self-reported wheezing. Variables were included in the final multivariate model if any of the following conditions were met: (1) there was a significant association between the predictor variable and the dependent variable in the respective univariate analysis, (2) there was an a priori hypothesis that an effect may exist based on plausible biological mechanisms, or (3) the multivariate analysis was controlled for age and sex. When significance in the multivariate model was defined at $P = .05$, only outdoor animal exposure was associated with a significantly lower risk of atopy, and no variables were significantly associated with wheezing. In the final multivariate model, when significance was instead defined at $P = .10$, different patterns of association emerged for the 2 outcomes. For self-reported wheezing, only the use of mud floors was associated with significantly lower risk. In contrast, for atopy, mud floor use was no longer statistically significant; however, animal exposure (indoor and outdoor) and exclusive breastfeeding in early infancy were associated with significantly lower risk. During stepwise creation of the multivariate model, no evidence of interaction was noted. Rural locale was not a significant predictor in the final multivariate model for either outcome.

DISCUSSION

This study shows a significantly lower prevalence of reported wheezing in rural children in India, confirming the trend that has been previously reported for India^{7–9} and other developing countries.^{2–5} This study further found a lower prevalence of reported rhinitis and objective atopic sensitization in rural Indian children, which has not been previously reported, to our knowledge. Atopy studies of children living in other rural areas of developing nations (ie, Ethiopia¹⁰ and Kenya¹¹) reveal a prevalence range for atopy (ie, ≥ 1 positive SPT result)

Table 2. Rural vs Urban Comparison of Animal Exposure and Cow Dung Use

	Rural group (n = 50)	Urban group (n = 50)	P value
Animal exposure, %			
Indoor			
Dogs, cats, and birds	80	4	<.001
Farm animals	12	0	.01
Mice/rats	44	4	<.001
Outdoor			
Dogs, cats, and birds	78	24	<.001
Farm animals	58	10	<.001
Mice/rats	12	2	.11
Any animal exposure, indoors at current time, %	84	6	<.001
Any animal exposure, outdoors at current time, %	80	28	<.001
Cow dung use, %			
Regular daily	94	0	<.001
Ceremonial	2	14	<.001
None	4	86	<.001

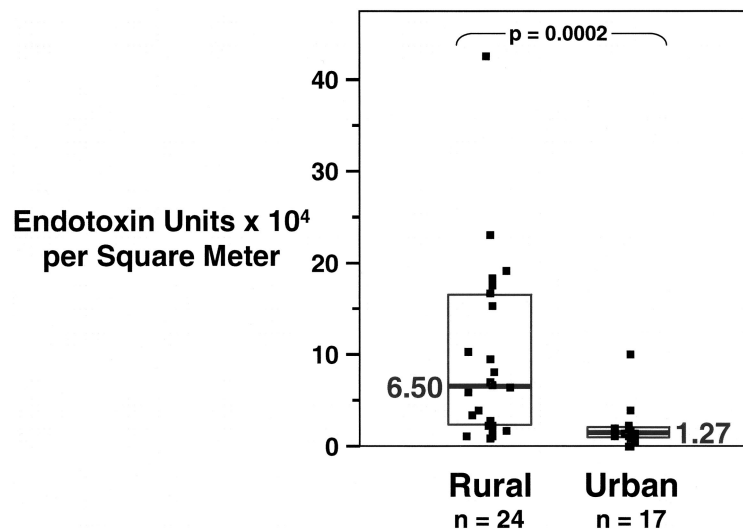


Figure 1. Comparison of endotoxin loads in rural vs urban homes in southern India. Endotoxin units were measured using a *Limulus* ameocyte lysate assay. Endotoxin load was calculated per square meter of collection area in each home. Points depict the endotoxin load for individual households; the lower, upper, and middle lines of the box plots indicate the 25th quantiles, the 75th quantiles, and the medians (with numerical values), respectively. $P < .001$ by Wilcoxon rank sum test.

of 11% to 41% and sensitization to dust mite of 16% to 25%, to cockroach of 0% to 15%, and to cat of 0% to 17%. For comparison, the present study observed an atopy prevalence in rural Indian children of 36% and sensitization to dust mite of 18%, to cockroach of 20%, and to cat of 12%. Although these studies are not methodologically identical, this comparison suggests that the prevalence of atopy and sensitization to common allergens in children raised in rural India, Ethiopia, or Kenya might not differ to a significant degree.

Previous studies in other developing countries have highlighted several environmental factors to account for observed rural-urban differences in asthma and atopic sensitization. In such rural locales, the microbial exposures associated with less atopy have primarily been those of the gastrointestinal tract: parasitic infestation^{12–14} or certain patterns of gut flora

in early childhood.^{15–17} Regarding animal exposure in similar locales, a study⁶ in Nepal observed that keeping cattle inside the home at night was related to a lower risk of asthma. Similarly, in rural homes in Kenya and Guinea-Bissau, less allergen sensitization or exercise-induced bronchospasm is associated with living with animals.^{5,18} It is interesting that the associations of animal contact^{19–24} and endotoxin exposure^{25–29} with less atopy, less atopic dermatitis, and less asthma have recently and consistently been reported for children raised in modern metropolitan and farming communities. The protective association of such childhood exposures seems to be widespread.

Table 3. Rural vs Urban Comparison of Self-reported Atopic Diseases and SPT Sensitivity

	Rural group (n = 50)	Urban group (n = 50)	P value
Self-reported diseases, %			
Rhinitis ever	22	42	.03
Wheeze ever	8	30	.005
Eczema	10	0	.02
Allergen sensitization, %			
House dust mite	18	44	.005
Cockroach	20	40	.03
Cat	12	16	NS
Cattle	6	12	NS
Dog	6	10	NS
At least 1 positive SPT result	36	58	.03

Abbreviations: NS, not significant; SPT, skin prick test.

Table 4. Univariate Analysis of Atopy and Self-reported Wheezing

	Unadjusted odds ratio (90% CI)	
	Atopy	Wheezing
Rural locale	0.4 (0.2–0.9)	0.2 (0.1–0.7)
Mud flooring	0.5 (0.2–1.2)	0.1 (0.01–0.6)
Cow dung use ever	0.4 (0.2–1.0)	0.2 (0.1–0.7)
Preschool attendance	3.2 (1.4–7.3)	4.7 (1.4–15.4)
Hepatitis B vaccination	2.5 (1.1–6.0)	1.3 (0.5–3.7)
Any animal exposure, indoors at current time	0.3 (0.1–0.7)	0.2 (0.05–0.6)
Any animal exposure, outdoors at current time	0.3 (0.2–0.8)	0.4 (0.2–1.2)
Exclusively breastfed >6 mo	0.3 (0.1–0.6)	0.3 (0.1–1.0)
No. of siblings	0.8 (0.6–1.1)	0.6 (0.3–1.0)
Current smoker in house	0.4 (0.2–0.8)	0.3 (0.1–1.1)
Measles vaccination	0.6 (0.3–1.5)	0.6 (0.2–1.8)
Female sex	0.8 (0.4–1.8)	0.6 (0.2–1.8)
Family history (atopy)	0.7 (0.3–1.6)	1.7 (0.6–4.7)

Abbreviation: CI, confidence interval.

Table 5. Sex- and Age-Controlled Multivariate Analysis of Atopy and Self-reported Wheezing

	Adjusted odds ratios (90% CI)	
	Atopy	Wheezing
Rural locale	2.3 (0.4–14.8)	2.0 (0.2–15.9)
Mud flooring ever	1.2 (0.4–3.5)	0.1 (0.02–1.0)
Any animal exposure, indoors at current time	0.2 (0.05–0.9)	0.6 (0.1–3.2)
Any animal exposure, outdoors at current time	0.3 (0.1–0.8)	1.1 (0.4–3.6)
Exclusively breastfed >6 mo	0.2 (0.1–0.5)	0.6 (0.2–2.2)
Cow dung use ever	2.4 (0.5–10.8)	0.6 (0.1–3.0)
Family history (atopy)	0.5 (0.2–1.2)	1.3 (0.5–3.5)
No. of siblings	1.0 (0.8–1.4)	0.8 (0.4–1.6)
Current smoker in house	0.5 (0.2–1.3)	0.7 (0.2–2.6)

Abbreviation: CI, confidence interval.

In the present study, many of these environmental factors, although apparently important in the univariate analysis (Table 4), did not ultimately show a significant association in the multivariate analysis (Table 5). Indeed, because of the small number of study patients in our randomly selected cohort, significance in the multivariate analysis was defined at $P = .10$ to detect more subtle trends. In multivariate analysis, 2 different patterns of association emerged for 2 respective outcomes. For self-reported wheezing, only the use of mud floors was associated with significantly lower risk, whereas for atopy, animal exposure (indoor and outdoor) and exclusive breastfeeding were associated with significantly lower risk.

The different patterns of association for the 2 outcomes can be attributed to several possible factors. First, different pathophysiologic processes may exist for wheezing and atopic SPT sensitization. Alternatively, breastfeeding may confer protection in a causal pathway independent of the other 2 factors—mud floors and animal exposure—which themselves may share a common final pathway. This common pathway could involve differential endotoxin levels and microbial exposure. In farming communities, such differential effects have been reported for endotoxin (ie, toll-like receptor 4 [TLR-4] ligand), which has been associated with less hay fever, atopic sensitization, and atopy-associated wheezing.²⁹ In comparison, among these children, exposure to the TLR-2 ligand muramic acid was associated with less wheezing but not less atopy.³⁰ Rural homes in India have also been reported to have greater microbial DNA (ie, TLR-9) content, which has been associated with a stronger regulatory (ie, interleukin 10) immune response.³¹ The limited dust sampling for endotoxin measurement in these homes revealed higher dust endotoxin loads in rural homes, which is consistent with this theory, but a more detailed investigation of these TLR ligands in these settings is needed. Home animal exposures and mud flooring may provide subtle but important differences in microbial

exposures (exemplified in these differences in TLR ligands) and subsequent atopy and wheezing outcomes.

There is controversy regarding the relationship between breastfeeding and the development of asthma and atopy, as various prospective studies have shown a protective effect,³² no effect, or even an increased risk of disease.³³ In the present study, 74% of the rural children were exclusively breastfed for more than 6 months vs only 20% of the urban children. In the multivariate analysis, exclusive breastfeeding was the strongest independent predictor of lower atopy, but it was not significantly related to self-reported wheezing. Our results support the literature that reports a protective role for breastfeeding against childhood asthma and atopy.³⁴ One possible explanation is that protective breastfeeding may be affected by microbial exposures of the mothers, which may transmit an immune protective effect through breast milk. In support of this possibility, lactobacillus supplementation to breastfeeding mothers is associated with less atopic dermatitis in their children,^{33,34} as well as more transforming growth factor $\beta 2$ in their breast milk, which is believed to be a mediator of immune regulatory responses.³⁵ Furthermore, breast milk can contain high quantities of soluble CD14, an enhancer binding protein for endotoxin.³⁶ Indeed, traditional Indian lifestyles include the frequent (almost daily) consumption of home-made yogurt made by fermentation with *Lactobacillus*-containing cultures. This dietary routine typically begins with children at approximately 9 months of age. It is intriguing to speculate that this aspect of the traditional Indian diet may improve the quality of breast milk from an atopy- and asthma-protective standpoint.

The results of the present study highlight the importance of household environmental factors. As reported previously herein, there were significant differences in rural vs urban homes (Figs 2 and 3). Most of the rural homes were constructed of mud walls with thatched or tiled roofs and had mud flooring on which cow dung was rubbed daily. Many rural homes had no separate rooms for sleeping; the same space was used for sleeping, dining, and socializing and also to keep animals indoors. In contrast, most urban homes had brick and mortar construction with a tiled or cement roof, separate bedrooms, and a cement floor. Cow dung was not used regularly in any urban home, except for ceremonial purposes, whereas it was used on a regular basis for domestic purposes in most rural homes (Fig 4). The routine use of cow dung on mud flooring is believed to reduce dustiness, to reduce insects such as termites and ants, and to have beneficial antiseptic properties. This suggests that close living with animals, mud flooring and walls, and regular domestic cow dung use in rural homes may increase the load of microbial components such as endotoxin and other TLR ligands, thereby reducing the subsequent risk of asthma and atopy, independent of the household occupation and possibly independent of community-wide environmental factors.

Other environmental factors include passive tobacco smoke exposure, outdoor automobile pollution (greater in urban areas), kitchen fuel sources (generally propane gas in



Urban house



Rural house

Figure 2. Representative outside views of urban (A) and rural (B) housing.

urban areas vs dried wood and cow dung cakes in rural areas), and water and sewage facilities (generally greater in urban areas). In the univariate analysis, a current smoker in the home was associated with a lower likelihood of atopy; however, this effect was not seen in the multivariate analysis, suggesting that this association was probably due to other factors associated with a rural lifestyle. Otherwise, our study did not investigate the contribution of these factors to differential atopy rates, but they are important areas of future research. An interesting demographic phenomenon evident in India is the presence of “rural pockets of living” located within large urban metropolitan areas. A study comparing rural pockets and other urban sites could control for common

locale-wide environmental factors, such as air pollution and water and sewage facilities.

There were several limitations of this study. First, the small number of individuals analyzed led to low statistical power and increased risk of type II error. Although 164 children were initially enrolled, only 100 children randomly selected from each household were considered for final analysis to eliminate potential confounding due to same-household linkages. However, this decreased the power of the study. Second, the urban children in the study were of a middle class background; urban children of the upper or lower socioeconomic classes of urban Mysore were not represented in this study. Third, the limited number of household dust endotoxin



Urban bedroom



Rural household (interior)

Figure 3. Representative inside views of urban (A) and rural (B) housing.



Figure 4. Domestic uses of cow dung in rural households.

determinations made it impossible to include endotoxin levels in the statistical analysis. This disallows us from commenting directly on the relationship between endotoxin and atopy and asthma prevalence in the study sample. Fourth, we lack data on parasitic infestation; thus, we cannot comment on this potentially important confounder.^{10,37} In addition, the wheezing and rhinitis outcomes were self-reported using the modified International Study of Asthma and Allergies in Children questionnaire and are subject to the shortcomings of self-reported outcomes. In comparison, our measure of atopy via SPT by an investigator who was kept unaware of the interview questionnaire findings was an objective measure. It is possible that this difference in the objectivity of these measures may also partially account for the observed different patterns of association.

In conclusion, in a rural-urban cohort in India we found that close animal contact and exclusive breastfeeding are associated with lower atopic sensitization and that living on mud floors is associated with lower rates of self-reported wheezing. This study adds to the growing body of evidence that exposure to animals and associated environmental microbial components in childhood reduces the development of atopy and asthma and serves as one of the first demonstrations of this relationship in India. Future research with more patients and using a prospective study design would best serve our understanding of this interesting story of the hygiene theory of asthma and atopy.³⁸

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