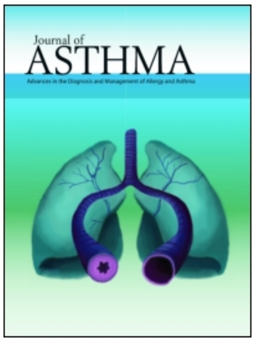

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Rurality, asthma and allergy in preschoolers.

Differences between preschoolers with asthma and allergies in urban and rural environments

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ABSTRACT

Objective: The influence of living in a rural or urban environment on asthma and allergic diseases in school-children provide conflicting results; moreover none of these studies included exclusively preschoolers. The aim of the present study was to evaluate if recurrent wheezing preschoolers from rural or urban areas differ in asthma, allergic diseases, and atopy. Methods: A cross-sectional-study in Rafaela,

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Argentina, on 143 preschoolers with recurrent wheezing from rural and urban settings was performed (2010-2012). Diagnosis of asthma (by positive asthma predictive index [API]), allergic diseases (rhinitis, dermatitis), and atopy (by skin prick test [SPT], peripheral blood eosinophils, and serum total IgE) were evaluated. Results: Preschoolers from rural settings had significantly higher prevalence of vaginal delivery, longer breastfeeding, earlier onset of wheezing, more parental smoking, siblings, shared a bedroom, and more exposure to chemicals used in plant fumigation or farm animals, and unpasteurized milk consumption, in comparison to preschoolers living in urban setting. In contrast, preschoolers from urban areas had significantly higher prevalence of parental history of allergy, positive skin prick test, and positive API. After multivariate analysis adjusting for covariates, maternal smoking (OR = 3.44), and positive SPT (OR = 5.57) significantly increase the risk of asthma diagnosis (positive API); in contrast, living in rural setting (OR = 0.04), and having more siblings (OR = 0.51) decrease their risk. Conclusions: Recurrent wheezing preschoolers from rural areas had a significant inverse odds of being diagnosed with asthma (type-2 inflammation) when compared to those from urban areas. Exposure to farm animals and consumption of unpasteurized milk might have a role.

Keywords

asthma predictive index, atopy, dairy farm, dermatitis, preschoolers, rhinitis, rural and urban residence, unpasteurized-milk, wheeze.

INTRODUCTION

Asthma is a frequent chronic disease in young children [1]. Asthma prevalence continues to rise in schoolchildren from developing countries, in contrast to those from industrialized countries, where a plateau has been reached [2]. For example, in Latin America, asthma prevalence in children aged 6-7 and teenagers aged 13-14 was found to be 8.4-37.6% and 11.6 - 27.3%, respectively. This high variability in asthma prevalence might be partly explained by differences in socioeconomic status, environmental exposures (e.g. second-hand tobacco smoke and air pollution), psychosocial stress, obesity, healthcare access, migration, and urban environment growth [3]. Moreover, in contrast to what happened with children from Central Europe, where the farming environment was found to be protective against asthma and allergic diseases [4], comparison data from studies about urban and rural environments in Latin America has provided conflicting results [5-7]. Moreover, there have been no studies on asthma or allergic diseases exclusively performed in preschoolers comparing the differences between those living in urban areas and those living in rural areas.

An important limitation of existing studies reporting asthma in children < 5 years of age is that different wheezing phenotypes coexist in them. Therefore, asthma diagnosis without lung function testing becomes a challenge for pediatricians, resulting in a wide variety of treatment approaches [8]. The latest guidelines for asthma diagnosis consider the number of wheezing episodes experienced, in addition to the existence of family and personal histories of atopy [9]. However, using the extensive common tool for asthma diagnosis (e.g. Asthma Predictive Index (API)) [10] might help to better predict asthma at preschool age, since the original API is the only algorithm that at the moment fulfills all the clinical prediction rules (e.g. development, validation/updating, impact, and implementations) [11].

The study of asthma in this specific age group (< 5 years) would be of great importance for a number of reasons. First, the burden of disease is greatest in preschoolers with a significantly higher proportion of emergency department visits, more hospitalizations, more sleep disturbances and more limitation of family activities/play than older children [12, 13]. Second, the irreversible impairment in lung function may occur during the preschool period, suggesting a window of opportunity to perhaps prevent irreversible damage [14]. It is possible that the repeated and cumulative lung injury caused by various respiratory infections (e.g., rhinovirus, respiratory syncytial virus, etc.) that are frequent at this age may be causal or important intercurrent factors affecting lung growth and asthma persistence.

Argentina, one of the most urbanized countries in Latin America, with over 90% of the population living in cities undergoing major changes in western lifestyle [15] and with a 13.6% asthma prevalence [2] provides us with a unique opportunity to test the hypothesis of how recurrent wheezing preschoolers from urban and rural areas differ in their diagnosis of asthma (using the API tool), allergic diseases, and atopy.

METHODS

A cross-sectional study was carried out between January 2010 and January 2012 in the city of Rafaela (Santa Fe Province, Argentina). We included a convenience sample of <5 years of age children with recurrent wheezing (≥3 episodes in the previous year, as documented by their pediatrician) [11] in outpatient clinics located in downtown Rafaela and in five surrounding rural areas. All the children enrolled were referred to a pediatric pulmonology clinic in Rafaela, and they were seen there by a single asthma specialist (DAM), who checked if they would meet the inclusion criteria. They were then divided into two groups according to the geographic area they lived in: urban or rural. Those from the "urban area" were from downtown Rafaela and had good sanitary conditions (safe drinking water and bathroom at home). Also, 100% of them had private health insurance and an annual per-capita family income of about US$ 50,000. In contrast, those from the "rural area" were from small farm towns (~50 kilometers in the periphery of Rafaela), where dairy farm activities and grain harvests are common. Most of them had

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poor sanitary conditions, only 20% had private health insurance, and their annual per-capita family income was around US$ 12,000. Those with preexisting cystic fibrosis, gastroesophageal reflux, lower airway recurrent infections, bronchopulmonary dysplasia, tuberculosis, heart and congenital pulmonary malformations, foreign body aspiration, primary ciliary dyskinesia, and/or immunodeficiency were excluded from the study.

A detailed questionnaire was completed and collected at enrollment (see supplemental material). This questionnaire included information about demographic characteristics, parental history of asthma and allergic diseases, use of antibiotics by the mother during pregnancy and breastfeeding, exposure to farm animals, pets and tobacco, cooking and heating appliances, bedroom ventilation index (windows´ meters/bedrooms' cubic meters x 100), fumigation near the house (less than 100 meters away), wheezing episodes, use of antibiotics during the first year of life, and assistance to day care centers. Anthropometric measurements and stigmata of allergic rhinitis and atopic dermatitis were also recorded during the enrollment process. Allergic rhinitis was diagnosis based on the presence of three out of four cardinal signs according to national guidelines [16]. Atopic dermatitis was diagnosed when children met major and minor criteria according to European guidelines [17].

A peripheral blood sample was obtained the same day the admission questionnaire was completed in order to assess eosinophil counts and IgE measurements done by electrochemiluminescence (COBAS 6000, Roche Diagnostics® Mannheim, Germany) and expressed in IU per ml. A skin prick test (SPT) was performed using antigens for common local allergens found in the city (Alergo Pharma®, Buenos Aires, Argentina) to study the children, and with positive (histamine) and negative (saline) for the controls. For children under two years of age, the SPT set contained eight aeroallergens (D. farinae, D. pteronyssinus, grain dust, cat, dog, Alternaria, Hormodendrum and Penicillium), and two food allergens (egg and peanut). In children over two years of age, three additional antigens were included (pollens from trees and

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grasses, and cow's milk). A SPT was considered positive if, after waiting for 15 minutes and subtracting the negative control value, the wheal size diameter of at least one allergen was ≥ 3 mm. Finally, the stringent original API [10] was determined in all preschoolers. An API was considered positive if at least one major criterion (parental MD asthma or MD eczema) or two minor criteria (MD allergic rhinitis, wheezing apart from colds or eosinophilia >4%) were present [10].

Written informed consent was obtained from all patients' parents or guardians, and all children assented to their enrollment. The study was authorized by the Institutional Research Ethics Board of Clínica Universitaria Reina Fabiola, which is part of the School of Medicine of the Universidad Católica de Córdoba, in Argentina.

Statistical analysis

We compared the preschoolers coming from urban areas with those from rural settings. For categorical variables, absolute frequencies and percentages with a 95% confidence interval (95% CI) were obtained, and chi-square tests were performed. The distribution of numerical variables was evaluated using the Shapiro-Wilk test, and those with normal distribution were presented as mean ± standard deviation (SD), otherwise as medians with interquartile range (IQR). Differences were compared using the Fischer exact test, t-test or Mann-Whitney U test, as appropriate.

A multivariable logistic regression model was constructed. Positive API was the dependent variable. All variables incorporated in the bivariate analysis were included as independent variables in the logistic model using the stepwise method. These variables were: sex, age, weight, height, birth weight, gestational age, type of delivery, duration of breastfeeding, parental history of allergies, paternal smoking, maternal smoking, number of siblings, number of people sleeping in the same bedroom, ventilation index, pets at home, antibiotics, age of wheezing onset, serum total IgE, and SPT. The variables were entered by the software if $p<0.05$, and removed if $p >0.1$. In order to avoid collinearity, parental history of asthma,

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atopic dermatitis, allergic rhinitis and % of eosinophils in peripheral blood counts were excluded from the model. Other factors associated with rural settings (fumigation, contact with farm animals, and unpasteurized milk consumption) were not introduced into the model in order to avoid confounding effects. Statistical significance was set at $p < 0.05$. The analysis was performed using the MedCalc Statistical v.13.3.3 software (Ostend, Belgium).

RESULTS

One hundred forty-three out of 150 children were enrolled in the study. Seven children did not fulfill the inclusion criteria or refused blood sampling. Of the remaining 143 children, their ages ranged from 9 to 60 months (38.8±1.3 m), 75 (52.5%) were males, 82 (57.3%) lived in an urban surrounding, and 61 lived in a rural setting. The results of the bivariate analysis documented no differences in terms of age, weight, height, birth weight or gestational age between the groups. However, children from the rural areas had significantly more prevalence of vaginal deliveries and a longer duration of exclusive breastfeeding than the children from urban settings. Moreover, the onset of their wheezing episodes was significantly earlier (Table 1). Also, children from rural areas had significantly more siblings, a higher number of people per bedroom, more parental smoking, more unpasteurized milk consumption, more farm animals, and more exposure to air fumigation with chemicals than those living in urban areas. In contrast, children from the urban settings had a significantly greater parental history of allergies than those from rural areas (Table 1). There were no significant differences between both groups in their cooking and heating systems, antibiotics consumption by the mother, and assistance to daycare centers (data not shown).

The proportion of positive SPTs to at least one allergen was significantly higher in urban than in rural children (Table1). However, peripheral blood eosinophils counts and serum total IgE were not significantly different between both groups (Table 1). Finally, the proportion of positive APIs was significantly higher in children from urban areas (Table 1).

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A multivariate analysis model was explored (Table 2). In this model, maternal smoking (OR = 3.44), and a positive SPT (OR = 5.57) significantly increased the risk of asthma diagnosis (positive API); in contrast, living in a rural setting (OR = 0.04) and having more siblings (OR = 0.51) decreased the risk.

DISCUSSION

The study carried out on 143 Argentinian preschoolers from rural and urban areas with recurrent wheezing showed differences in their asthma diagnosis and their atopy characteristics. After a multivariate analysis, it was observed that maternal smoking and positive SPT significantly increased the risk of asthma diagnosis (positive API), while living in a rural setting and having more siblings decreased the risk. Remarkably, living in rural areas was the strongest factor inversely related to having positive API, decreasing their odds by ~96%.

To our knowledge, this is the first study exclusively done in preschoolers that compares the characteristics of recurrent wheezing in rural and urban settings. We have confirmed our hypothesis that those who live in rural settings have significantly fewer asthma diagnoses by positive APIs (type-2 inflammation) than those from rural settings. As mentioned before, one of the reasons of a high morbidity among preschoolers is that the diagnosis of asthma without lung function testing is very challenging, thus postponing the treatment or leading to a wide variation of treatment approaches without solid evidence [8]. Recently, a randomized trial found that the probability of a best response to daily inhaled corticosteroids (ICS) was further increased in preschoolers with both aeroallergen sensitization and blood eosinophil counts of ≥ 300/mL. In these preschoolers with type-2 inflammation, daily ICS use was associated with more asthma control days and fewer exacerbations compared with montelukast or intermittent ICS [18]. However, for preschoolers without type 2 inflammation, no clear treatment preference has been found yet [18]. Therefore, we could speculate that daily ICS might be the preferred

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treatment among recurrent wheezing preschoolers living in urban settings (asthma with type-2 inflammation).

Previous studies comparing schoolchildren in rural and urban settings showed that those living in rural or farm areas had less asthma, fewer positive SPTs, and less allergic diseases (rhinitis and dermatitis) than those in urban areas, following the hygiene hypothesis. In rural regions of the Alpine area (Austria, Germany, and Switzerland), children living in farms have been shown to have 20% to 57% lower risk of asthma, hay fever and atopic dermatitis than those not living in farms [19]. Moreover, farm living has been associated with 55% decreased odds of current wheezing among non-atopic children in the same areas [20]. Similar results were shown in a study in Poland [21], where farming activities are slightly different from those in the Alpine area, as well as in studies comparing farming environments or rural lifestyle and allergic diseases in China [22], Korea [23], Australia [24], and Canada [25].

However, residing in rural areas of Latin America has not always been associated with a reduced risk of wheezing, asthma or allergic diseases in schoolchildren [3]. For example, a similar prevalence of current wheezing, atopy, or eczema between schoolchildren living in urban and rural areas was reported in a tropical area of Ecuador, but those living in urban settings had increased odds of current rhinitis [5]. In contrast, a study of schoolchildren living in high altitude urban and rural areas of Oropeza, in Bolivia, showed that those in rural areas had significantly 50% decreased odds of current wheezing, and 60% decreased odds of severe rhinoconjunctivitis, compared to those from urban areas [6]. Similarly, in Valdivia, Chile, those schoolchildren living in rural residences had a notably 60% reduction of their asthma symptoms and a 70% reduction of rhinoconjunctivitis when compared to schoolchildren from urban settings [26]. Finally, a recently reported study on teenagers of Cordoba Province, in Argentina, showed that residing in a rural setting or have been residing for >5 years is associated with reduced odds of recurrent wheezing and allergic rhinoconjunctivitis [7].

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Another interesting finding from our study was that, even though the consumption of unpasteurized milk and the presence of farm animals were significantly more prevalent in rural settings than in urban ones, they were not introduced into the model to avoid confounding effects. Contact with livestock and animal feed/shed, consumption of unprocessed cow's milk, and microbial (bacterial or fungal) exposures may explain the beneficial effects of a farming environment as protective from allergic diseases [4].

In a mouse model, chronic exposure to low-dose endotoxin [lipopolysaccharide] or farm dust prevented house dust mite (HDM)--induced allergic airway inflammation, reduced levels of interleukin (IL)-5 and IL-13, and activated dendritic cells. Compared to controls, lipopolysaccharide-treated mice had lower serum IgE to HDM, decreased airway responsiveness, and fewer eosinophils in bronchoalveolar lavage [27]. Moreover, a Finnish cohort study found that farming environments affect the function of dendritic cells and induce a T-regulatory and Th1-skewed phenotype, promoting immune tolerance [28, 29]. A recent study in schoolchildren from Amish and Hutterite farms in America reported that, despite similar genetic ancestries and lifestyle between the groups, the prevalence of asthma and allergic sensitization was 4 to 6 times lower in the Amish, whereas median endotoxin levels in an Amish house dust was 6.8 times higher [30]. In a mouse model of experimental allergic asthma, the intranasal instillation of dust extract from Amish but not Hutterite homes significantly inhibited airway hyperreactivity and eosinophilia [30].

The present study has also some limitations. First, as a cross-sectional study, we cannot adequately assess causality or temporal cause-effect relationships. Second, since this study were done in children with recurrent wheezing, the results are generalizable only to populations of children with recurrent wheezing. Third, factors like air pollution and cotinine measurements, diet during pregnancy,

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and diet at child's early life were not specifically addressed. However, we cannot exclude their potential role as mediators or confounders in our results.

In summary, our findings suggest that the environment of residence leads to two different recurrent wheezing patterns among preschoolers. Those living in an urban area started their wheezing episodes significantly later, and had more positive APIs and more atopy (positive SPTs) than those living in rural areas. After a multivariate analysis, living in rural areas was found to be the most potent inverse factor (~96% less chance) for being diagnosed with asthma by a positive API (type-2 inflammation). However, more longitudinal studies still need to be carried out in order to examine the mechanisms underlying the protective effects of rural environments in Argentina and in other low-middle income countries.

Funding Source

None.

Conflict of Interests

The authors declare having no conflicts of interest to disclose relevant to this article

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QUESTIONNAIRE FOR PARENTS		
Name and Last name ………………………………………………….…………Code:………..….		
Date of birth: …………………. Age: ……..….. Address: …………………..………………….		
City/town: ………………………………………. Phone number: ……..……………………..…….		
1) Personal History		
Controlled pregnancy:	Gestational age:	Type of delivery:
Birth weight:	Age of 1st wheezing episode:	
Number of wheezing per month:	Recurrent cough:	
Recurrent wheezing last year:	Choking crisis:	Number of hospitalizations due to wheezing exacerbation:
Wheezing with our colds (viral):	Atopic dermatitis (AD):	Rhinitis:
Intestinal parasites:	Antibiotics intake per year:	Paracetamol (acetaminophen) intake:
Day care center:		
2) Family History		
Father with asthma:	Mother with asthma:	Grandparents with asthma:
Father with allergy:	Mother with allergy:	Grandparents with allergy:
Number of siblings:	Siblings with asthma:	Siblings with allergy:
Father smoker:	Mother smoker:	Both smokers:
Father with AD:	Mother with AD:	Siblings with AD:
Mother smoked during pregnancy:	Mother smoked when breastfeeding:	Both:
Mother took antibiotics	During pregnancy:	When breastfeeding:
3) Nutritional History		
Breastfeeding duration:	Infant formula:	
Unpasteurized cow's milk:	Pasteurized whole milk:	
Month of solid food introduction:	Fruits:	

Vegetables:	Nuts:	
Weight: Height:	Head circumference:	
Growth percentile from exam:		
4) House Details		
Urban environment:	Rural environment:	
Brick-built house:	Adobe house, hut:	
Number of bedrooms:	People per bedroom:	
Good ventilation:	Poor ventilation:	
Wood-burning cook stove:	Wood-burning stove:	
Dirt floor:	Sheet metal roof:	
Number of pets: Dog:	Cat: Others:	
Nearby air fumigation with chemicals:		
5) Tests performed		
* Complete questionnaire:		
* Complete cytologic evaluation:	IgE:	Eosinophils:
* Skin prick test:		

Table 1. Demographic and clinical characteristics of children from urban and rural settings in the bivariate model.

	Urban n = 82	Rural n = 61	OR (95%CI)	p-value
Male	47 (57.3)	28 (45.9)	1.6 (0.8-3.2)	0.18
Age (mo)	40.5 ± 15.2	36.4 ± 1.6	NA	0.12
Weight (kg)	16.8 ± 4.5	15.9 ± 4.8	NA	0.22
Height (cm)	98.4 ± 13.2	96.1 ± 12.9	NA	0.29
Birth weight (gr)	3231 ± 419	3232 ± 632	NA	0.99
Gestational age (wk)	39 [38-39]	39 [38-40]	NA	0.15
Vaginal birth	21 (25.6)	32 (52)	0.3 (0.1-0.7)	0.01
Duration of breastfeeding (mo)	6.5 [3-13]	12 [6-20.5]	NA	0.01
Parental history of asthma	31 (37.8)	19 (31.1)	1.3 (0.6-2.9)	0.41
Parental history of allergies	66 (80.5)	37 (60.6)	2.7 (1.2-61)	0.01
Paternal smoking	17 (20.7)	25 (40.9)	0.4 (0.2-0.8)	0.01
Maternal smoking	10 (12.2)	12 (19.6)	0.3 (01-0.9)	0.00
Number of siblings	1 [0-1]	1 [0.7-2]	NA	0.02
No. people sleeping/bedroom	2 [2-3]	3 [2-4]	NA	<0.01
Ventilation index	12.3±0.5	11.1±0.6	NA	0.17
Fumigation	0 (0)	57 (93.4)	NA	<0.01
Farm animals	2 (2.4)	44 (72.1)	0.009 (0.001-0.04)	<0.01
Pets at home	58 (70.7)	24 (39.3)	0.6 (0.2-1.4)	0.19
Unpasteurized milk consumption	5 (6)	39 (63.9)	0.04 (0.01-0.11)	<0.01
Antibiotics (# cycles per yr)	7 [4-10]	7 [5-12]	NA	0.46
Age wheezing onset (mo)	6 [5-10]	5 [3-7.2]	NA	0.01

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Atopic dermatitis	22 (26.8)	13 (23.3)	1.3 (0.6-2.9)	0.29
Allergic rhinitis	59 (71.9)	31 (50.8)	1.4 (0.7-2.8)	0.37
% eosinophils peripheral blood	3 [2-5]	3 [1-5]	NA	0.39
# eosinophils peripheral blood	293.5 [184-475]	270 [103.7-490]	NA	0.47
Serum total IgE (IU/ml)	160 [42-310]	78.4 [38.4-317]	NA	0.56
Positive skin prick test (SPT)	32 (39)	12 (19.7)	2.6 (1.2-6.2)	0.01
Positive API	77 (93.9)	30 (49.2)	15.9 (5.3-56.1)	<0.01

Numbers were expressed as n (%), mean ± SD, or median [interquartile range] where applicable. API = asthma predictive index; NA = not applicable, OR = odds ratio.

Table 2. Multivariate logistic regression model to predict asthma diagnosis (positive API).*

Variable	Coefficient	SE	p	aOR	95% CI
Maternal smoking	1.23	0.62	<0.05	3.44	1.02 to 11.11
Rural setting	-3.11	0.60	<0.01	0.04	0.01 to 0.14
Siblings	-0.67	0.24	<0.01	0.51	0.31 to 0.83
Positive skin prick test	1.72	0.70	<0.01	5.57	1.40 to 22.23
Constant	-1.02				

*In order to avoid collinearity, parental history of asthma, atopic dermatitis, allergic rhinitis and % of eosinophils in peripheral blood counts were excluded from the model. Other factors associated with rural settings (fumigation, contact with farm animals, and unpasteurized milk consumption) were not introduced into the model to avoid confounding effects.

API = asthma predictive index, SE = standard error, aOR = adjusted odds ratio, CI = confidence interval.