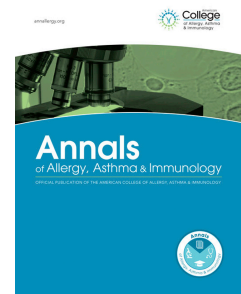


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Risk factors for wheezing in primary health care settings in the tropics

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Risk factors for wheezing in primary health care settings in the tropics

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Conflict of interest

DLL reports personal fees outside the submitted work from Amstrong, Astrazeneca, Boehringer Ingelheim, DBV Technologies, Grunenthal, GSK, Mylan, Menarini, MSD, Novartis, Pfizer, Novartis, Sanofi, UCB. And grants from Sanofi, Astrazeneca, Novartis, UCB, GSK, TEVA, Boehringer Ingelheim, Chiesi. None of the other authors declared any conflict of interest.

No local requirement exists for IRB approval for anonymously reported surveys

According to local legislation no IRB approval nor written informed consent is needed for anonymous surveys, in which no data are revealed that could track details back to individuals. We add a letter of the Chair of the Board of Lawyers of the State of Tabasco in this respect, that was written in reply to our specific request.

19-05-0264R3

Background : The "International Study of Wheezing in Infants (EISL)", is a cross-sectional, population-based study, based on ISAAC. "(Http // www.isaac.auckland.ac.nz). It uses a validated questionnaire on early wheezing and risk/protective factors.

Objective: To apply EISL questionnaire regarding wheezing events in 0-12months' infants with/without atopic background searching for risk-factors in the tropics. Methods Population: toddlers coming in for check-up or 12-months' vaccination in primary health care clinics of a tropical city. Apart from child factors (i.e. day-care attendance) we evaluated home factors (i.e. air-conditioning, bathroom, carpet, >6 persons, pollution) and mother's factors (i.e. education level, employment, cellphone). Data analysis was descriptively and case-control, with as cases atopic (AW) or nonatopic (NAW) wheezing children versus healthy controls. Wheezing-associated factors were evaluated using multivariate analysis, adjusted for the relation of AW/NAW with factors, significant in prior univariate analysis.

Results : 999 included toddlers. Any wheeze: 31.3%, recurrent wheeze (≥ 3 episodes):

12.1%. Major risk-factors for AW (OR;95%CI): smoking (11.39;2.36-54.99), common cold <3mo of life (3.72;2.59-5.36), mold (3.48;2.28-5.30), kitchen indoors (2.40;1.27-

4.54) and pets (1.69;1.09-2.62); breastfeeding was almost protective. For NAW common cold and pets were risk-factors, but caesarean section (0.44;0.23-0.82), >1 sibling (0.33;0.18-0.61) and breastfeeding >3m (0.50;0.28-0.91) protective.

Conclusion : Wheezing is a health care burden. We found potential new risk-factors for AW, some possibly unique for tropical climates. We suggest testing several hypotheses: could early AW be reduced in the tropics attacking mold growth? enhancing cooking place ventilation? keeping pets outside? Or by postponing day-care attendance till after four months and avoiding (passive) smoking during pregnancy?

1 Introduction (2859 words)

2 Wheezing in infants is a condition that brings a burden on families, caretakers and the
3 health care system, as a child in distress causes anguish among family members and
4 dyspnea in a preschooler often leads to emergency department attendance. Moreover, it
5 has been shown that viral induced wheeze might lead to asthma later in life.(1) Yet the
6 prevalence of wheezing in infants had not been studied in many populations, until the
7 "International Study of Wheezing in Infants (EISL, by its Spanish abbreviation)" was
8 developed by a multinational group of investigators, aiming to investigate the frequency
9 of wheezing in infants and to determine associated risk and protective factors. To that
10 means investigators developed a standardized questionnaire for the interviews
11 (QE-EISL – phase 1) and validated it.(2) This made comparisons between different
12 populations possible(3-5) and repeating the questionnaire 5 years later allowed
13 observation of trends in early wheeze and its treatment.(6)
14 As the questionnaire included questions related to the presence of skin allergies in the
15 infant and rhinitis, asthma and skin allergies in first degree relatives, children could be
16 divided in clinically atopic and non-atopic. As such, the objective of the here presented
17 study was to determine the prevalence of wheezing and recurrent wheezing during the
18 first year of life in a tropical climate and to determine risk and protective factors for
19 atopic and non-atopic wheezing children, using the EISL questionnaire. We
20 hypothesized we might find some new risk factors in our tropical environment.

21

22 Methods

23 The "International Study of Wheezing in Infants (EISL, by its Spanish abbreviation)" is
24 multinational, multicenter, population-based study in random samples of infants in
25 which a validated questionnaire(2, 7) was used, partially based on the "International
26 Study of Asthma and Allergies in Childhood (ISAAC). "(Http //
27 www.isaac.auckland.ac.nz). The questionnaire includes questions about wheezing
28 during the first year of life, its frequency and severity, and questions about possible risk
29 or protective factors.

Population and questionnaires

This study is designed to evaluate a representative sample of 1000 children in the first year of life living in Villahermosa, a tropical city in the south of Mexico, between January 2009 and October 2011. According to the National Institute of Statistics we estimate at there lived about 12,500 children < 1 year that moment in Villahermosa.(8) As such, recruitment has been carried out in primary health care clinics, linked to the large Regional Pediatric Hospital. During a routine visit of the preschoolers to the clinic for their regular check-up or for the administration of the 12-months' vaccination, the parents or guardians of the children were invited to fill in the EISL questionnaire. The questions related to wheezing and events that occurred during the first 12 months of the child's life. The field workers were well-instructed and supervised primary health care personnel and medical residents. Verbal informed consent was obtained before conducting the survey interview. According to local legislation no ethics committee approval nor written informed consent is needed in surveys with anonymous reporting of results.

The study consists of two parts. The first part is descriptive, documenting the characteristics of the enrolled children as one whole group, specifically focusing on the presence of wheezing episodes, their frequency and severity, but also on child's characteristics, and household specifics. The second part of the study is a case-control analysis on risk-factors for wheezing, either in children with an atopic or a non-atopic background. In this analysis, the cases are the children who reported wheezing and we used our healthy toddlers as controls. In the analyses the wheezing children were subdivided in atopic and non-atopic wheezers, as defined below.

Definitions

Wheezing has been defined as having a positive answer to the question: 'Has your baby had wheezing in the chest during the first 12 months of life?' In this context, interviewing health workers were allowed to explain to the mothers what was meant by wheezing.

Atopic wheeze: we categorize the wheezing as atopic if there was also a positive answer to either of these questions: 'Does your baby have an allergic skin disease (e.g. allergic dermatitis, eczema, etc.) at this moment, or did he suffer from it during this first year of life?' or 'Has your baby first degree family members with asthma, allergic rhinitis or skin allergy?'. In a final analysis, we used a more stringent definition of atopy,

considering as atopic only those children with both, atopic dermatitis and first-degree family members with one or more of the atopic conditions mentioned above, to see if there would be any change in the associated risk or protective factors. Results of this analysis can be found in the online repository.

NON-atopic wheezing was defined as a positive answer to the wheezing question, but a negative answer to the other two above-mentioned questions on personal or family atopy.

The *healthy children* were defined as those who never had wheezed, and who moreover had no personal nor family history of atopic dermatitis, asthma or allergic rhinitis, i.e. healthy, non-atopic children.

The *risk or protective factors* possibly related to wheeze, as obtained with the validated EISL questionnaire we used in our study are 1) whether the child was born by caesarean section, 2) frequency of consumption of products rich in fat and/or sugar such as yoghurt, flan or junk-food as chips, etc. and soft drinks, 3) common colds during the first 3 months of life, 4) day-care attendance, 5) other child conditions (full vaccination schedule, breastfeeding more than 3 months), 6) mother's conditions (smoking during pregnancy) 7) several housing conditions (electric/gas/wooden stove, air conditioning, carpets, indoor bathroom, indoor visible mold, number of siblings, people living in the home, pets at birth, pets currently and level of outdoor pollution) and 8) social conditions (mobile phone, mother's educational level and employment).

Data analysis

The first analysis is descriptive, concerning the presence of wheeze in preschool infants, its frequency and severity and the treatment options received. Also, the frequency in the whole study population of the risk-factors and protective factors shall be described.

In the second part of this study, a case-control analysis has been developed. Crude associations between the risk / protection factors and the response variables (atopic wheezing and non-atopic wheezing) were calculated using Odds Ratio (OR) and 95% confidence intervals (95% CI). We have used generalized linear models with a family of binomial distribution and a link logit for multivariate analysis, in which all factors with significance ($p < 0.05$) and those close to significance in the univariate analysis, have been included as explanatory variables; and atopic wheezing and non-atopic wheezing were considered as dependent variables (Stata v10.0, Collage Station, TX, USA).

96 Results

97 *Descriptive analysis (total population)*

98 A total of 1001 questionnaires have been returned, 999 fulfilling the inclusion criteria.
99 During the first year of life 31.3% reported ever wheeze and 123 (12.1%) children
100 among them reported recurrent wheeze. Most of the children with wheeze at 0-12
101 months (53%) started the first episode between 2-4 months (Figure 1). Emergency
102 health care utilization of the children with ever wheeze was considerable: ER visit 41%
103 and hospitalization 13%. Sixty-three percent of the caretakers reported inhaled
104 bronchodilator being part of the treatment and 40% inhaled corticosteroids, while a
105 quarter received leukotriene receptor antagonists at some point. Apart from the
106 wheezing, the caregivers indicated 8.7% of all children had suffered from pneumonia
107 and two thirds of them were hospitalized with a diagnosis of (bronco)pneumonia. The
108 diagnosis of pneumonia was rare in children without wheezing (2.4%); among the
109 children with wheezing ever, a history of pneumonia was more common in those with
110 physician diagnosed asthma, Odds Ratio 2.13 (CI 1.21-2.78), as opposed to non-
111 asthmatic children (26 versus 14%, $p<0.01$). Daycare attendance was started somewhat
112 earlier in children who reported wheezing (Figure 2).

113 In the total study population, atopic conditions in first degree family members were
114 asthma (24%), allergic rhinitis (22%) and skin allergies (14%). Half of the mothers had
115 finished secondary or high school and a third was employed at the time of the interview.
116 Twenty-six percent of the children received breastfeeding beyond six months.

117 Housing conditions varied from those of lower to those of middle class: most had 0-2
118 siblings, 3-6 people living in the house, all had a gas-stove, 90% inside the house, 78%
119 a complete bathroom and half had air-conditioning, but carpeting was rare. 168/703
120 (23.9%) of the caregivers reported mold at home. A third had a pet at the child's birth or
121 at the moment of interview and a third agreed they lived in a moderate-to-severely
122 polluted area.

123 *Case-control study: wheezing children versus healthy controls*

124 Figure 3 shows the flowchart for the case-control part. Of the wheezing children 256
125 had atopic wheeze and 57 non-atopic wheeze, while 309 were pure healthy controls.
126 Table 1 shows the frequency of factors possibly associated with wheezing for atopic

children and healthy controls and aOR's (95% CI) with univariate analysis. Table 2 shows the same for non-atopic wheezing children.

As a next step, we conducted a multivariate analysis, correcting for factors that had been significant in the univariate analysis, see tables 1 and 2, bold figures. Table 3 shows the results of the multivariate analysis for both, factors for which a significant association with wheezing could be confirmed and for those without significant association. Here, several factors that seemed to be of importance in the univariate analysis were rejected as risk or protective factors in the multivariate analysis. As such, in the multivariate analysis we did not find anymore an association between atopic wheeze and sex, caesarean section, attending day-care, presence of siblings, mother's employment status or pets at the moment of birth. Six or more persons in the house was almost a risk factor and breast feeding showed a trend for being a protective factor, as confidence intervals just crossed unity.

For non-atopic wheeze, we rejected the following potential risk or protective factors: sex, attending day-care, indoors cooking or mold or pets, presence of siblings or number of persons in the house or mother's employment status. For smoking during pregnancy, the number of smoking mothers in these groups was too small to be analyzed.

When conducting the same analyses with a more stringent definition of atopic wheeze (see methods), cooking indoors and pets at home did just not reach significant association anymore. For the rest, no major changes were found, see e-table.

Discussion

We here present the results of an epidemiologic, questionnaire-based study on wheezing in the first year of life among toddlers visiting primary healthcare centers in a city in the tropical south-eastern region of Mexico. A third of the toddlers presented at least one episode of early wheeze (<12 months of age), resulting in ER visits in 41% and hospital admission in 13% of them, reflecting a considerable burden on health-care utilization and costs. Moreover, 12.3% of our toddlers presented recurrent wheeze. In the multivariate analysis of the case-control part of our study we found risk and protective factors associated with early wheeze being partly different for atopic versus non-atopic children. In both groups breast-feeding for more than three months was an (almost) protective factor, while common colds before three months of age and mother's smoking during pregnancy were risk factors. Pets at home also seemed to be a risk

factor, be it 'at the moment of the study' for the atopic children and at birth for non-atopic children. However, in atopic children having mold at home, an indoor kitchen or more than five inhabitants are all risk factors. Meanwhile, in the non-atopic children two further factors were protective, reducing the OR of wheezing: being borne by caesarean section and having siblings.

The strength of the here presented study is its sample size, which enables us to reveal with an acceptable degree of certainty, the reality of wheezing in low-middle class pre-school children in a tropical city, an environment in which not many reliable studies on this concept have been published. Also, the use of a validated questionnaire for wheezing and related factors, with a validated translation into local language(2) augments the reliability of our findings. Finally, the multivariate analysis with a more stringent definition of atopy, showing almost the same results add to this study's power.

The weaknesses of our study are the use of a questionnaire, which might lead to recall bias. Also, the caregiver's reporting of mold in the home does not forcefully reflect the real situation in the subject's house and it might even be that a mother in an atopic family is more cautious in detecting mold, biasing the results. Further, the selection of children attending primary health care facilities for check-up or vaccination. This sample selection leaves out the two extremes of children: on the one hand the upper-class preschoolers who only see a private physician and on the other hand those who are not seen by health care personnel, generally the extremely poor population.

The same questionnaire has been used in other studies in Europe and Latin America. By comparing our results with theirs we can observe some differences. Although the most important independent risk factors for wheeze, found in these studies and confirmed in our population, were having a cold during the first 3 months of life and mother's smoking during pregnancy, we did not find male gender(9) was a risk factor. Attending day care seemed also to be a risk factor for our atopic wheezing children in the univariate evaluation, but did not hold stand in the multivariate analysis, as the 95% CI included unity. As for the protective factors, we confirmed exclusive breastfeeding for >3 months in both our wheezing groups, but university studies of the mother did not protect our children from wheeze, as it did do in other parts of Latin America.(3)

In line with findings from other places where risk factors for wheezing have been investigated we confirm that an early age of the first common cold(4) and maternal smoking during pregnancy are risk factors. The explanation for these two is clear. Viral infections are the prime cause for wheezing in infants.(10) In this sense, if early viral

infections can lead to the development of atopy and asthma is still open to discussion.(1) Actually the risk of developing asthma is greatest for young children who wheeze with rhinovirus infections.(11) Very recently DNA methylation changes at several genomic sites in chromosomal regions previously linked to asthma (e.g. the SMAD3 gene promoter) have been found to be associated with atopic asthma after rhinovirus-induced wheezing,(12) but this may be different for RSV.(13) Day-care attendance from very young age on could augment the frequency of these very early viral infections and with them the early onset (see figures 1 and 2) of the first wheezing episode. Furthermore, it has been known for long that maternal smoking is one of the most important prenatal determinants of reduced neonatal lung function.(14) Remarkably, our study results suggest the possible presence of unique factors related to an augmented frequency of early wheezing in a tropical population, especially in atopic children: indoor mold, indoor cooking and pets' presence. We would like to postulate the following as possible explanations. Firstly, Villahermosa has a tropical climate: 24% of all our households reported the presence of mold. Mold exposure during infancy has been demonstrated to be a predictor of potential asthma development,(15) partly because of allergic sensitization, but also because of volatile substances and other toxins that molds secrete.(16, 17) Similarly, a small study in Costa Rica concluded that lifetime childhood asthma prevalence (LCAP) was related to relative moldiness index values in floor dust, but not to dust mite allergen concentrations in bed dust (positive in 90% of all homes, but not different in homes with high versus low LCAP).(18) With the fast evolving climate changes of the past decades, larger areas of the world could be exposed to more humid climates in the future, thus augmenting the infant population at-risk as has been documented for other pediatric disorders recently.(19) On the other hand, though, caregiver's verbal report of indoor mold could be biased and should be confirmed and quantified in future trials. Secondly, cooking indoors was related to atopic wheeze; all our families reported the use of gas-stoves that could lead to indoor pollution, as the cooking-living-sleeping spaces are often within the same area. Finally, related to pets being a risk-factor for wheezing: in our population pets are rarely cleaned, thus emanating considerable quantities of pet-allergens and house dust, the latter being a cause of allergy in almost 90% of patients with allergic rhinitis living in Villahermosa(20). A causal relationship of these facts warrants further investigated. In this context, our findings open two research questions we suggest should be tested with interventional trials: 1) does improving indoor air quality (attacking mold,

enhancing ventilation) reduce the frequency and severity of wheezing in atopic infants in tropical settings? 2) does postponing day-care attendance till > 4 months reduce the frequency and severity of wheezing in infants?.

In conclusion, infant wheezing in the tropics is frequent and most have their first episode between 2-4 months. Over forty percent of them is brought to the emergency department, conveying a considerable burden on health-care costs. Moreover, our study points at several possible risk- and protective factors related to the presence of wheezing, some of them not reported previously. Population-based studies like this one are of importance, as they could lead to hypotheses that should be tested, and at the end could lead to recommendations for public health. .

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Figure legends:

Figure 1: Age (months) of the first wheezing episode in infants who reported at least one wheezing episode between birth and 12 months of age. N = 311. Note: though there were 313 children with ever wheeze, there were 2 missing answers to this question on time of first wheeze.

Figure 2: Cumulative age of start of daycare attendance in months for children with (n = 70) and without (n = 133) wheezing. For wheezing infants: Median 5 months, (P5-P95: 1-12m). For non-wheezing infants: Median 7 months, (P5-P95: 2-12m). Statistically significant differences in ratio of children attending day-care between groups with chi-square (Pearson): * p<0.05, ** p<0.01

Figure 3: Flow diagram showing how the whole study population can be subdivided into children who fulfilled the criteria for atopy and with at least one wheezing episode, non-atopic children with at least one wheezing episode, healthy children with no atopy nor wheezing at all and a rest group with non-specific replies to some respiratory

326 symptom questions. Two questionnaires of the 1001 were hardly completed at all and
327 thus excluded from further analysis.

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Table 1. Study population characteristics and univariate association between early atopic wheeze and possible risk/protection factors: Children with early wheezing and clinical atopy -personal or first-degree family- versus healthy children (no wheezing nor atopy).

Items with significant differences between groups in bold.

	Atopic wheeze (N=256)		Healthy (no wheezing nor atopy) (N=390)		Odds ratios (OR) and 95% confidence intervals (95% CI)	
	n	(%)	n	(%)	OR	CI 95%
Male	147	(57.4)	223	(57.2)	1.01	(0.73,1.39)
Smoking during pregnancy	16	(6.3)	2	(0.5)	12.93	(2.95,56.74)
Cesarean section	131	(51.2)	189	(48.5)	1.14	(0.83,1.57)
Fast food						
Never	81	(31.6)	119	(30.5)	1	(unity, no CI)
Once a month	19	(7.4)	38	(9.7)	0.73	(0.40,1.36)
Once a week	132	(51.6)	203	(52.1)	0.96	(0.67,1.37)
Every day	22	(8.6)	30	(7.7)	1.08	(0.58,2.00)
Common cold < 3mo of life	159	(62.1)	131	(33.6)	3.31	(2.38,4.60)
Attending daycare	58	(22.7)	60	(15.4)	1.63	(1.09, 2.43)
Having air-conditioning	128	(50.0)	190	(48.7)	1.07	(0.78,1.47)
Carpet at home	18	(7.0)	28	(7.2)	0.99	(0.53,1.82)
Having bathroom at home	199	(77.7)	293	(75.1)	1.20	(0.82,1.75)
Cooking indoors, in the house	236	(92.2)	335	(86.1)	2.11	(1.20,3.70)
Cellphone	190	(74.2)	276	(70.8)	1.23	(0.86,1.75)
Mother's level of education						
Basic	38	(14.8)	55	(14.1)	1	(unity, no CI)
Incomplete middle level	80	(31.3)	133	(34.1)	0.87	(0.53,1.43)
Complete middle and superior level	136	(53.1)	202	(51.8)	0.97	(0.61,1.55)
Mold	99	(38.7)	57	(14.6)	3.73	(2.55,5.44)
Complete vaccination schedule	231	(90.2)	352	(90.3)	1.08	(0.63,1.87)
Siblings (>1)	217	(84.8)	281	(72)	1.10	(0.97,1.25)
Persons in the house (>=6)	69	(27.0)	58	(14.9)	1.17	(1.06,1.28)
Breastfeeding >3m	142	(55.5)	256	(65.6)	0.66	(0.48,0.92)
Mother's employment	88	(34.4)	106	(27.2)	1.43	(1.02,2.01)
Pollution	136	(53.1)	199	(51.0)	1.11	(0.81,1.52)
Pets at birth	107	(41.8)	129	(33.1)	1.47	(1.06,2.04)
Pets at the moment	103	(40.2)	102	(26.2)	1.92	(1.37,2.70)

Table 2. Study population characteristics and univariate association between early non-atopic wheeze and possible risk/protection factors for children with no clinical atopy - personal or first-degree family-: wheezers versus non-wheezers. Items with significant differences between groups in bold.

	Non-atopic wheeze (N=57)		Healthy (no wheezing nor atopy) (N=390)		Odds ratios (OR) and 95% confidence intervals (CI 95%)	
	n	%	n	%	OR	CI 95%
Male	29	(50.9)	223	(57.2)	0.77	(0.44, 1.35)
Smoking during pregnancy	0	(0.0)	2	(0.5)	Numbers too small	
Cesarean section	17	(29.8)	189	(48.5)	0.45	(0.25, 0.82)
<i>Fast food</i>						
Never	19	(33.3)	119	(30.5)	1	(unity, no CI)
Once a month	8	(14.0)	38	(9.7)	0.80	(0.42, 1.51)
Once a week	26	(45.6)	203	(52.1)	1.32	(0.53, 3.25)
Every day	4	(7.0)	30	(7.7)	0.83	(0.26, 2.64)
Common cold < 3mo of life	32	(56.1)	131	(33.6)	2.53	(1.44, 4.45)
Attending daycare	6	(10.5)	60	(15.4)	0.65	(0.26, 1.57)
Having air-conditioning	20	(35.1)	190	(48.7)	0.57	(0.32, 1.02)
Carpet at home	0	(0.0)	28	(7.2)	Numbers too small	
Having bathroom at home	41	(71.9)	293	(75.1)	0.85	(0.45, 1.58)
Cooking indoors, in the house	43	(75.4)	335	(86.1)	0.49	(0.25, 0.96)
Cellphone	36	(63.2)	276	(70.8)	0.71	(0.40, 1.26)
<i>Mother's level of education</i>						
Basic	12	(21.1)	55	(14.1)	1	(unity, no CI)
Incomplete middle level	15	(26.3)	133	(34.1)	0.52	(0.23, 1.17)
Complete middle and superior level	30	(52.6)	202	(51.8)	0.68	(0.33, 1.42)
Mold	12	(21.1)	57	(14.6)	1.56	(0.78, 3.12)
Complete vaccination schedule	49	(86.0)	352	(90.3)	0.66	(0.29, 1.50)
Siblings (>1)	30	(52.6)	281	(72.1)	0.43	(0.24, 0.76)
Persons in the house (>=6)	13	(22.8)	58	(14.9)	1.69	(0.86, 3.33)
Breastfeeding >3m	29	(50.9)	256	(65.6)	0.54	(0.31, 0.95)
Mother's employment	10	(17.5)	106	(27.2)	0.57	(0.28, 1.67)
Pollution	35	(61.4)	199	(51.0)	1.53	(0.86, 2.70)
Pets at birth	29	(50.9)	129	(33.1)	2.09	(1.20, 3.67)
Pets at the moment	22	(38.6)	102	(26.2)	1.77	(0.99, 3.16)

NI: not included in de model, no significant variable.

Table 3. Multivariate analysis, with OR adjusted for the relation of atopic and non-atopic wheeze with factors, significant in the univariate analysis. Factors significantly associated with early wheeze in bold.

Children with atopic and non-atopic wheeze vs healthy children				
	Atopic wheeze		Non-atopic wheeze	
	OR	IC 95%	OR	IC 95%
Gender				
- Masculine	1.01	(0.70, 1.44)	0.87	(0.48,1.57)
Smoking during pregnancy	11.39	(2.36,54.99)	Numbers too small	
Cesarean section	Numbers too small		0.44	(0.23,0.82)
Common cold < 3mo of life	3.72	(2.59,5.36)	2.68	(1.46,4.91)
Cooking indoors, in the house	2.40	(1.27,4.54)	0.76	(0.37,1.56)
Mold	3.48	(2.28,5.30)	NI	
Siblings (>1)	NI		0.33	(0.18,0.61)
Persons in the house (>=6)	<i>1.10</i>	<i>(0.99,1.23)</i>	NI	
Breastfeeding >3m	<i>0.73</i>	<i>(0.50,1.06)</i>	0.50	(0.28,0.91)
Pets at birth	0.99	0.64,1.52	2.03	(1.11,3.70)
Pets at the moment	1.69	(1.09,2.62)	NI	
Attending daycare	1.34	(0.83,2.16)	NI	
Mother's employment	1.16	(0.78,1.72)	NI	

Italics: almost significant

NI: not included in the model, no significant variable in this group.

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Table 1. Study population characteristics and univariate association between early atopic wheeze and possible risk/protection factors: Children with early wheezing and clinical atopy -personal or first-degree family- versus healthy children (no wheezing nor atopy).

Items with significant differences between groups in bold.

	Atopic wheeze (N=256)		Healthy (no wheezing nor atopy) (N=390)		Odds ratios (OR) and 95% confidence intervals (95% CI)	
	n	(%)	n	(%)	OR	CI 95%
Male	147	(57.4)	223	(57.2)	1.01	(0.73,1.39)
Smoking during pregnancy	16	(6.3)	2	(0.5)	12.93	(2.95,56.74)
Cesarean section	131	(51.2)	189	(48.5)	1.14	(0.83,1.57)
Fast food						
Never	81	(31.6)	119	(30.5)	1	(unity, no CI)
Once a month	19	(7.4)	38	(9.7)	0.73	(0.40,1.36)
Once a week	132	(51.6)	203	(52.1)	0.96	(0.67,1.37)
Every day	22	(8.6)	30	(7.7)	1.08	(0.58,2.00)
Common cold < 3mo of life	159	(62.1)	131	(33.6)	3.31	(2.38,4.60)
Attending daycare	58	(22.7)	60	(15.4)	1.63	(1.09, 2.43)
Having air-conditioning	128	(50.0)	190	(48.7)	1.07	(0.78,1.47)
Carpet at home	18	(7.0)	28	(7.2)	0.99	(0.53,1.82)
Having bathroom at home	199	(77.7)	293	(75.1)	1.20	(0.82,1.75)
Cooking indoors, in the house	236	(92.2)	335	(86.1)	2.11	(1.20,3.70)
Cellphone	190	(74.2)	276	(70.8)	1.23	(0.86,1.75)
Mother's level of education						
Basic	38	(14.8)	55	(14.1)	1	(unity, no CI)
Incomplete middle level	80	(31.3)	133	(34.1)	0.87	(0.53,1.43)
Complete middle and superior level	136	(53.1)	202	(51.8)	0.97	(0.61,1.55)
Mold	99	(38.7)	57	(14.6)	3.73	(2.55,5.44)
Complete vaccination schedule	231	(90.2)	352	(90.3)	1.08	(0.63,1.87)
Siblings (>1)	217	(84.8)	281	(72)	1.10	(0.97,1.25)
Persons in the house (>=6)	69	(27.0)	58	(14.9)	1.17	(1.06,1.28)
Breastfeeding >3m	142	(55.5)	256	(65.6)	0.66	(0.48,0.92)
Mother's employment	88	(34.4)	106	(27.2)	1.43	(1.02,2.01)
Pollution	136	(53.1)	199	(51.0)	1.11	(0.81,1.52)
Pets at birth	107	(41.8)	129	(33.1)	1.47	(1.06,2.04)
Pets at the moment	103	(40.2)	102	(26.2)	1.92	(1.37,2.70)

Table 2. Study population characteristics and univariate association between early non-atopic wheeze and possible risk/protection factors for children with no clinical atopy - personal or first-degree family-: wheezers versus non-wheezers. Items with significant differences between groups in bold.

	Non-atopic wheeze (N=57)		Healthy (no wheezing nor atopy) (N=390)		Odds ratios (OR) and 95% confidence intervals (CI 95%)	
	n	%	n	%	OR	CI 95%
Male	29	(50.9)	223	(57.2)	0.77	(0.44, 1.35)
Smoking during pregnancy	0	(0.0)	2	(0.5)	Numbers too small	
Cesarean section	17	(29.8)	189	(48.5)	0.45	(0.25, 0.82)
<i>Fast food</i>						
Never	19	(33.3)	119	(30.5)	1	(unity, no CI)
Once a month	8	(14.0)	38	(9.7)	0.80	(0.42, 1.51)
Once a week	26	(45.6)	203	(52.1)	1.32	(0.53, 3.25)
Every day	4	(7.0)	30	(7.7)	0.83	(0.26, 2.64)
Common cold < 3mo of life	32	(56.1)	131	(33.6)	2.53	(1.44, 4.45)
Attending daycare	6	(10.5)	60	(15.4)	0.65	(0.26, 1.57)
Having air-conditioning	20	(35.1)	190	(48.7)	0.57	(0.32, 1.02)
Carpet at home	0	(0.0)	28	(7.2)	Numbers too small	
Having bathroom at home	41	(71.9)	293	(75.1)	0.85	(0.45, 1.58)
Cooking indoors, in the house	43	(75.4)	335	(86.1)	0.49	(0.25, 0.96)
Cellphone	36	(63.2)	276	(70.8)	0.71	(0.40, 1.26)
<i>Mother's level of education</i>						
Basic	12	(21.1)	55	(14.1)	1	(unity, no CI)
Incomplete middle level	15	(26.3)	133	(34.1)	0.52	(0.23, 1.17)
Complete middle and superior level	30	(52.6)	202	(51.8)	0.68	(0.33, 1.42)
Mold	12	(21.1)	57	(14.6)	1.56	(0.78, 3.12)
Complete vaccination schedule	49	(86.0)	352	(90.3)	0.66	(0.29, 1.50)
Siblings (>1)	30	(52.6)	281	(72.1)	0.43	(0.24, 0.76)
Persons in the house (>=6)	13	(22.8)	58	(14.9)	1.69	(0.86, 3.33)
Breastfeeding >3m	29	(50.9)	256	(65.6)	0.54	(0.31, 0.95)
Mother's employment	10	(17.5)	106	(27.2)	0.57	(0.28, 1.67)
Pollution	35	(61.4)	199	(51.0)	1.53	(0.86, 2.70)
Pets at birth	29	(50.9)	129	(33.1)	2.09	(1.20, 3.67)
Pets at the moment	22	(38.6)	102	(26.2)	1.77	(0.99, 3.16)

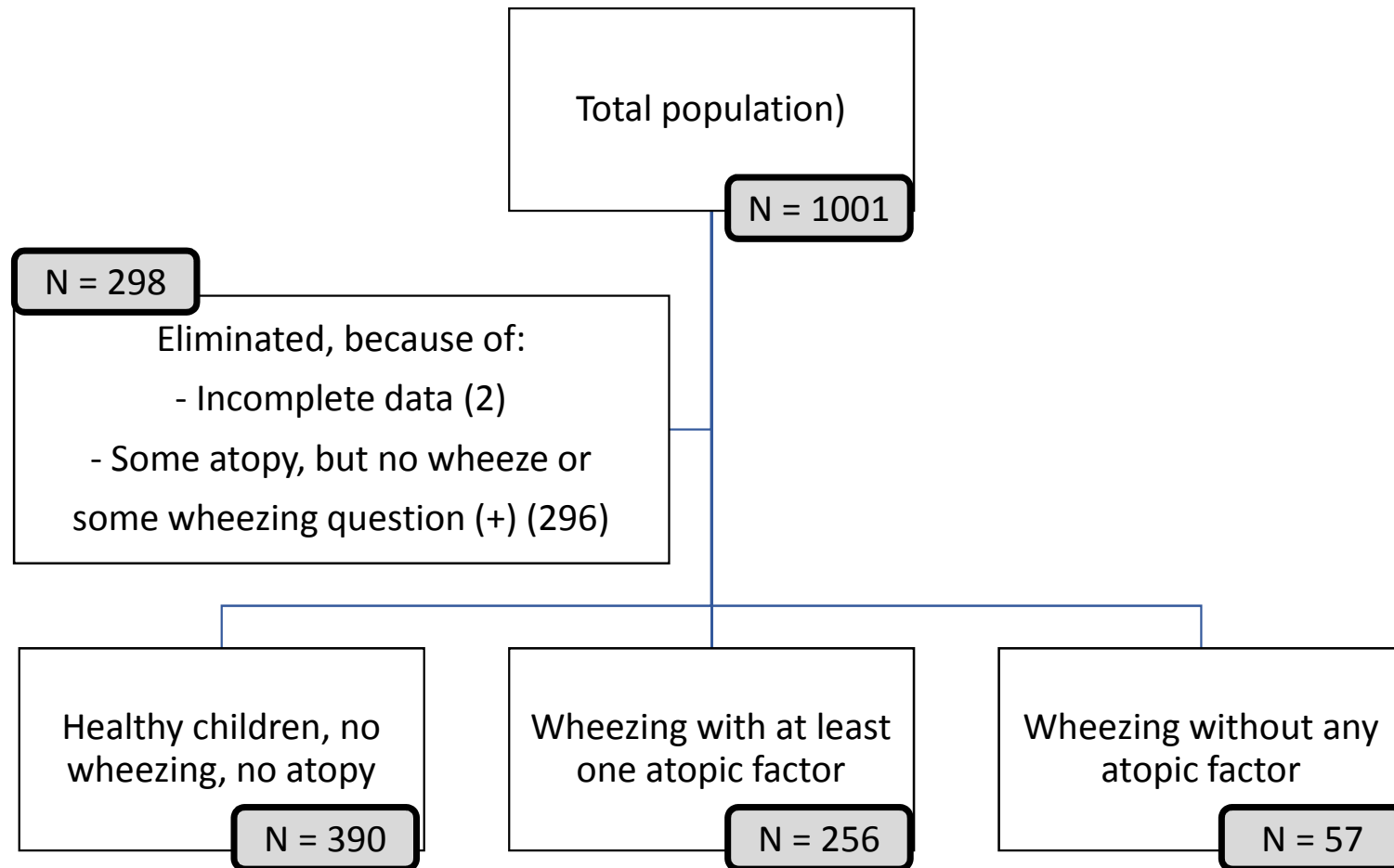
NI: not included in de model, no significant variable.

Table 3. Multivariant analysis, with OR adjusted for the relation of atopic and non-atopic wheeze with factors, significant in the univariate analysis. Factors significantly associated with early wheeze in bold.

Children with atopic and non-atopic wheeze vs healthy children				
	Atopic wheeze		Non-atopic wheeze	
	OR	IC 95%	OR	IC 95%
Gender				
- Masculine	1.01	(0.70, 1.44)	0.87	(0.48,1.57)
Smoking during pregnancy	11.39	(2.36,54.99)	Numbers too small	
Cesarean section	Numbers too small		0.44	(0.23,0.82)
Common cold < 3mo of life	3.72	(2.59,5.36)	2.68	(1.46,4.91)
Cooking indoors, in the house	2.40	(1.27,4.54)	0.76	(0.37,1.56)
Mold	3.48	(2.28,5.30)	NI	
Siblings (>1)	NI		0.33	(0.18,0.61)
Persons in the house (>=6)	<i>1.10</i>	<i>(0.99,1.23)</i>	NI	
Breastfeeding >3m	<i>0.73</i>	<i>(0.50,1.06)</i>	0.50	(0.28,0.91)
Pets at birth	0.99	0.64,1.52	2.03	(1.11,3.70)
Pets at the moment	1.69	(1.09,2.62)	NI	
Attending daycare	1.34	(0.83,2.16)	NI	
Mother's employment	1.16	(0.78,1.72)	NI	

Italics: almost significant

NI: not included in the model, no significant variable in this group.



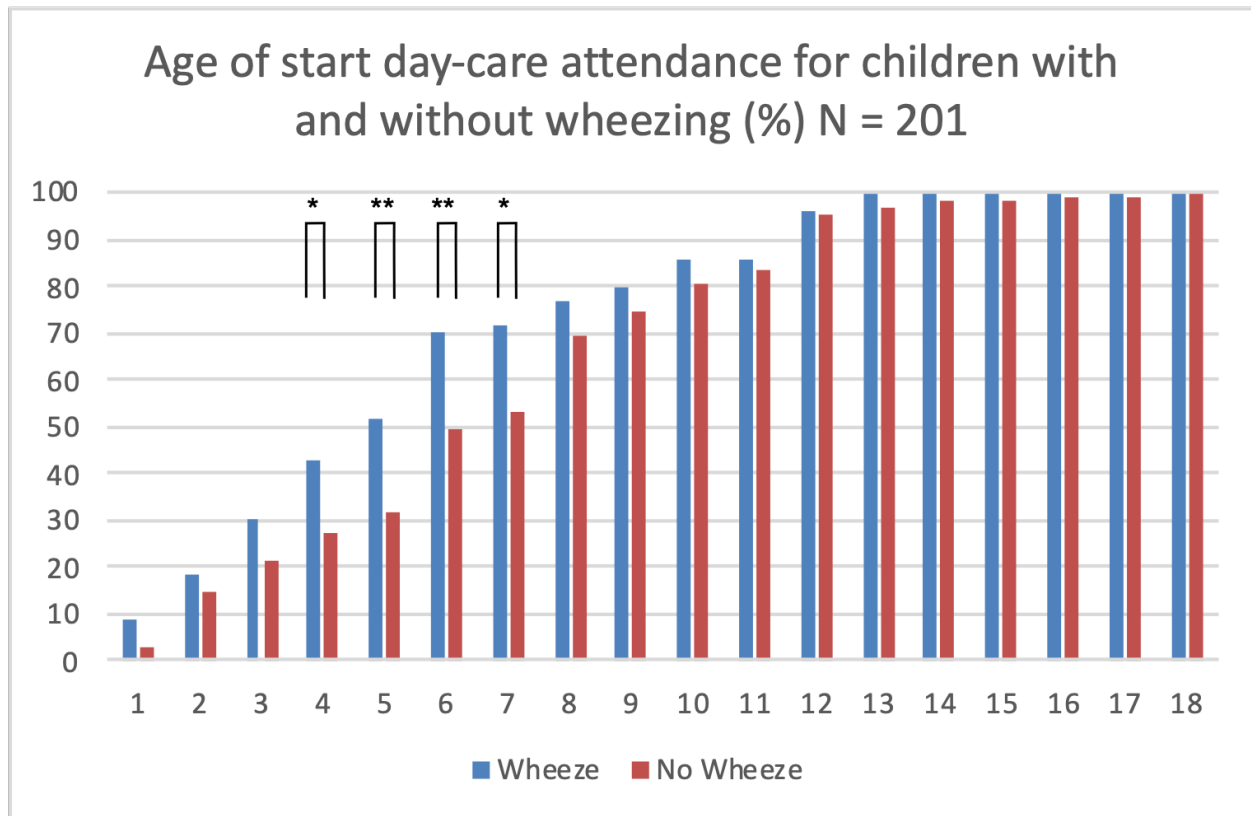


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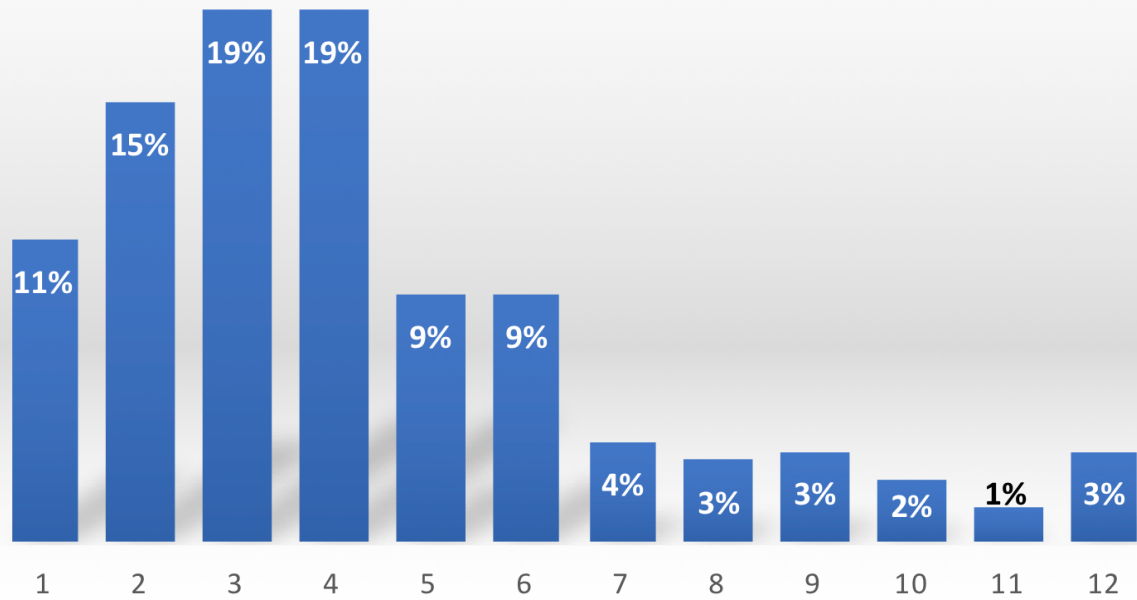
Figure 1: Age (months) of the first wheezing episode in infants who reported at least one wheezing episode between birth and 12 months of age. N = 311. Note: though there were 313 children with ever wheeze, there were 2 missing answers to this question on time of first wheeze.

Figure 2: Cumulative age of start of daycare attendance in months for children with (n = 70) and without (n = 133) wheezing. For wheezing infants: Median 5 months, (P5-P95: 1-12m). For non-wheezing infants: Median 7 months, (P5-P95: 2-12m). Statistically significant differences in ratio of children attending day-care between groups with chi-square (Pearson): * $p < 0.05$, ** $p < 0.01$

Figure 3: Flow diagram showing how the whole study population can be subdivided into children who fulfilled the criteria for atopy and with at least one wheezing episode, non-atopic children with at least one wheezing episode, healthy children with no atopy nor wheezing at all and a rest group with non-specific replies to some respiratory symptom questions. Two questionnaires of the 1001 were hardly completed at all and thus excluded from further analysis.

Age of first wheezing episode (months)

N = 311 pre-schoolers reporting ever wheezing



Introduction

Wheezing in infants is a condition that brings a burden on families, caretakers and the health care system, as a child in distress causes anguish among family members and dyspnea in a preschooler often leads to emergency department attendance. Moreover, it has been shown that viral induced wheeze might lead to asthma later in life.(1) Yet the prevalence of wheezing in infants had not been studied in many populations, until the "International Study of Wheezing in Infants (EISL, by its Spanish abbreviation)" was developed by a multinational group of investigators, aiming to investigate the frequency of wheezing in infants and to determine associated risk and protective factors. To that means investigators developed a standardized questionnaire for the interviews (QE-EISL – phase 1) and validated it.(2) This made comparisons between different populations possible(3-5) and repeating the questionnaire 5 years later allowed observation of trends in early wheeze and its treatment.(6) As the questionnaire included questions related to the presence of skin allergies in the infant and rhinitis, asthma and skin allergies in first degree relatives, children could be divided in clinically atopic and non-atopic. As such, the objective of the here presented study was to determine the prevalence of wheezing and recurrent wheezing during the first year of life in a tropical climate and to determine risk and protective factors for atopic and non-atopic wheezing children, using the EISL questionnaire. We hypothesized we might find some new risk factors in our tropical environment.

Methods

The "International Study of Wheezing in Infants (EISL, by its Spanish abbreviation)" is multinational, multicenter, population-based study in random samples of infants in which a validated questionnaire(2, 7) was used, partially based on the "International Study of Asthma and Allergies in Childhood (ISAAC). "(Http // www.isaac.auckland.ac.nz). The questionnaire includes questions about wheezing during the first year of life, its frequency and severity, and questions about possible risk or protective factors.

Population and questionnaires

This study is designed to evaluate a representative sample of 1000 children in the first year of life living in Villahermosa, a tropical city in the south of Mexico, between January 2009 and October 2011. According to the National Institute of Statistics we estimate at there lived about 12,500 children < 1 year that moment in Villahermosa.(8) As such, recruitment has been carried out in primary health care clinics, linked to the large Regional Pediatric Hospital. During a routine visit of the preschoolers to the clinic for their regular check-up or for the administration of the 12-months' vaccination, the parents or guardians of the children were invited to fill in the EISL questionnaire. The questions related to wheezing and events that occurred during the first 12 months of the child's life. The field workers were well-instructed and supervised primary health care personnel and medical residents. Verbal informed consent was obtained before conducting the survey interview. According to local legislation no ethics committee approval nor written informed consent is needed in surveys with anonymous reporting of results.

The study consists of two parts. The first part is descriptive, documenting the characteristics of the enrolled children as one whole group, specifically focusing on the presence of wheezing episodes, their frequency and severity, but also on child's characteristics, and household specifics. The second part of the study is a case-control analysis on risk-factors for wheezing, either in children with an atopic or a non-atopic background. In this analysis, the cases are the children who reported wheezing and we used our healthy toddlers as controls. In the analyses the wheezing children were subdivided in atopic and non-atopic wheezers, as defined below.

Definitions

Wheezing has been defined as having a positive answer to the question: 'Has your baby had wheezing in the chest during the first 12 months of life?' In this context, interviewing health workers were allowed to explain to the mothers what was meant by wheezing.

Atopic wheeze: we categorize the wheezing as atopic if there was also a positive answer to either of these questions: 'Does your baby have an allergic skin disease (e.g. allergic dermatitis, eczema, etc.) at this moment, or did he suffer from it during this first year of life?' or 'Has your baby first degree family members with asthma, allergic rhinitis or skin allergy?'. In a final analysis, we used a more stringent definition of atopy,

considering as atopic only those children with both, atopic dermatitis and first-degree family members with one or more of the atopic conditions mentioned above, to see if there would be any change in the associated risk or protective factors. Results of this analysis can be found in the online repository.

NON-atopic wheezing was defined as a positive answer to the wheezing question, but a negative answer to the other two above-mentioned questions on personal or family atopy.

The *healthy children* were defined as those who never had wheezed, and who moreover had no personal nor family history of atopic dermatitis, asthma or allergic rhinitis, i.e. healthy, non-atopic children.

The *risk or protective factors* possibly related to wheeze, as obtained with the validated EISL questionnaire we used in our study are 1) whether the child was born by caesarean section, 2) frequency of consumption of products rich in fat and/or sugar such as yoghurt, flan or junk-food as chips, etc. and soft drinks, 3) common colds during the first 3 months of life, 4) day-care attendance, 5) other child conditions (full vaccination schedule, breastfeeding more than 3 months), 6) mother's conditions (smoking during pregnancy) 7) several housing conditions (electric/gas/wooden stove, air conditioning, carpets, indoor bathroom, indoor visible mold, number of siblings, people living in the home, pets at birth, pets currently and level of outdoor pollution) and 8) social conditions (mobile phone, mother's educational level and employment).

Data analysis

The first analysis is descriptive, concerning the presence of wheeze in preschool infants, its frequency and severity and the treatment options received. Also, the frequency in the whole study population of the risk-factors and protective factors shall be described.

In the second part of this study, a case-control analysis has been developed. Crude associations between the risk / protection factors and the response variables (atopic wheezing and non-atopic wheezing) were calculated using Odds Ratio (OR) and 95% confidence intervals (95% CI). We have used generalized linear models with a family of binomial distribution and a link logit for multivariate analysis, in which all factors with significance ($p < 0.05$) and those close to significance in the univariate analysis, have been included as explanatory variables; and atopic wheezing and non-atopic wheezing were considered as dependent variables (Stata v10.0, Collage Station, TX, USA).

Results

Descriptive analysis (total population)

A total of 1001 questionnaires have been returned, 999 fulfilling the inclusion criteria. During the first year of life 31.3% reported ever wheeze and 123 (12.1%) children among them reported recurrent wheeze. Most of the children with wheeze at 0-12 months (53%) started the first episode between 2-4 months (Figure 1). Emergency health care utilization of the children with ever wheeze was considerable: ER visit 41% and hospitalization 13%. Sixty-three percent of the caretakers reported inhaled bronchodilator being part of the treatment and 40% inhaled corticosteroids, while a quarter received leukotriene receptor antagonists at some point. Apart from the wheezing, the caregivers indicated 8.7% of all children had suffered from pneumonia and two thirds of them were hospitalized with a diagnosis of (bronco)pneumonia. The diagnosis of pneumonia was rare in children without wheezing (2.4%); among the children with wheezing ever, a history of pneumonia was more common in those with physician diagnosed asthma, Odds Ratio 2.13 (CI 1.21-2.78), as opposed to non-asthmatic children (26 versus 14%, $p<0.01$). Daycare attendance was started somewhat earlier in children who reported wheezing (Figure 2).

In the total study population, atopic conditions in first degree family members were asthma (24%), allergic rhinitis (22%) and skin allergies (14%). Half of the mothers had finished secondary or high school and a third was employed at the time of the interview. Twenty-six percent of the children received breastfeeding beyond six months.

Housing conditions varied from those of lower to those of middle class: most had 0-2 siblings, 3-6 people living in the house, all had a gas-stove, 90% inside the house, 78% a complete bathroom and half had air-conditioning, but carpeting was rare. 168/703 (23.9%) of the caregivers reported mold at home. A third had a pet at the child's birth or at the moment of interview and a third agreed they lived in a moderate-to-severely polluted area.

Case-control study: wheezing children versus healthy controls

Figure 3 shows the flowchart for the case-control part. Of the wheezing children 256 had atopic wheeze and 57 non-atopic wheeze, while 309 were pure healthy controls. Table 1 shows the frequency of factors possibly associated with wheezing for atopic

children and healthy controls and aOR's (95% CI) with univariate analysis. Table 2 shows the same for non-atopic wheezing children.

As a next step, we conducted a multivariate analysis, correcting for factors that had been significant in the univariate analysis, see tables 1 and 2, bold figures. Table 3 shows the results of the multivariate analysis for both, factors for which a significant association with wheezing could be confirmed and for those without significant association. Here, several factors that seemed to be of importance in the univariate analysis were rejected as risk or protective factors in the multivariate analysis. As such, in the multivariate analysis we did not find anymore an association between atopic wheeze and sex, caesarean section, attending day-care, presence of siblings, mother's employment status or pets at the moment of birth. Six or more persons in the house was almost a risk factor and breast feeding showed a trend for being a protective factor, as confidence intervals just crossed unity.

For non-atopic wheeze, we rejected the following potential risk or protective factors: sex, attending day-care, indoors cooking or mold or pets, presence of siblings or number of persons in the house or mother's employment status. For smoking during pregnancy, the number of smoking mothers in these groups was too small to be analyzed.

When conducting the same analyses with a more stringent definition of atopic wheeze (see methods), cooking indoors and pets at home did just not reach significant association anymore. For the rest, no major changes were found, see e-table.

Discussion

We here present the results of an epidemiologic, questionnaire-based study on wheezing in the first year of life among toddlers visiting primary healthcare centers in a city in the tropical south-eastern region of Mexico. A third of the toddlers presented at least one episode of early wheeze (<12 months of age), resulting in ER visits in 41% and hospital admission in 13% of them, reflecting a considerable burden on health-care utilization and costs. Moreover, 12.3% of our toddlers presented recurrent wheeze. In the multivariate analysis of the case-control part of our study we found risk and protective factors associated with early wheeze being partly different for atopic versus non-atopic children. In both groups breast-feeding for more than three months was an (almost) protective factor, while common colds before three months of age and mother's smoking during pregnancy were risk factors. Pets at home also seemed to be a risk

factor, be it 'at the moment of the study' for the atopic children and at birth for non-atopic children. However, in atopic children having mold at home, an indoor kitchen or more than five inhabitants are all risk factors. Meanwhile, in the non-atopic children two further factors were protective, reducing the OR of wheezing: being borne by caesarean section and having siblings.

The strength of the here presented study is its sample size, which enables us to reveal with an acceptable degree of certainty, the reality of wheezing in low-middle class pre-school children in a tropical city, an environment in which not many reliable studies on this concept have been published. Also, the use of a validated questionnaire for wheezing and related factors, with a validated translation into local language(2) augments the reliability of our findings. Finally, the multivariate analysis with a more stringent definition of atopy, showing almost the same results add to this study's power.

The weaknesses of our study are the use of a questionnaire, which might lead to recall bias. Also, the caregiver's reporting of mold in the home does not forcefully reflect the real situation in the subject's house and it might even be that a mother in an atopic family is more cautious in detecting mold, biasing the results. Further, the selection of children attending primary health care facilities for check-up or vaccination. This sample selection leaves out the two extremes of children: on the one hand the upper-class preschoolers who only see a private physician and on the other hand those who are not seen by health care personnel, generally the extremely poor population.

The same questionnaire has been used in other studies in Europe and Latin America. By comparing our results with theirs we can observe some differences. Although the most important independent risk factors for wheeze, found in these studies and confirmed in our population, were having a cold during the first 3 months of life and mother's smoking during pregnancy, we did not find male gender(9) was a risk factor. Attending day care seemed also to be a risk factor for our atopic wheezing children in the univariate evaluation, but did not hold stand in the multivariate analysis, as the 95% CI included unity. As for the protective factors, we confirmed exclusive breastfeeding for >3 months in both our wheezing groups, but university studies of the mother did not protect our children from wheeze, as it did do in other parts of Latin America.(3)

In line with findings from other places where risk factors for wheezing have been investigated we confirm that an early age of the first common cold(4) and maternal smoking during pregnancy are risk factors. The explanation for these two is clear. Viral infections are the prime cause for wheezing in infants.(10) In this sense, if early viral

infections can lead to the development of atopy and asthma is still open to discussion.(1) Actually the risk of developing asthma is greatest for young children who wheeze with rhinovirus infections.(11) Very recently DNA methylation changes at several genomic sites in chromosomal regions previously linked to asthma (e.g. the SMAD3 gene promoter) have been found to be associated with atopic asthma after rhinovirus-induced wheezing,(12) but this may be different for RSV.(13) Day-care attendance from very young age on could augment the frequency of these very early viral infections and with them the early onset (see figures 1 and 2) of the first wheezing episode. Furthermore, it has been known for long that maternal smoking is one of the most important prenatal determinants of reduced neonatal lung function.(14) Remarkably, our study results suggest the possible presence of unique factors related to an augmented frequency of early wheezing in a tropical population, especially in atopic children: indoor mold, indoor cooking and pets' presence. We would like to postulate the following as possible explanations. Firstly, Villahermosa has a tropical climate: 24% of all our households reported the presence of mold. Mold exposure during infancy has been demonstrated to be a predictor of potential asthma development,(15) partly because of allergic sensitization, but also because of volatile substances and other toxins that molds secrete.(16, 17) Similarly, a small study in Costa Rica concluded that lifetime childhood asthma prevalence (LCAP) was related to relative moldiness index values in floor dust, but not to dust mite allergen concentrations in bed dust (positive in 90% of all homes, but not different in homes with high versus low LCAP).(18) With the fast evolving climate changes of the past decades, larger areas of the world could be exposed to more humid climates in the future, thus augmenting the infant population at-risk as has been documented for other pediatric disorders recently.(19) On the other hand, though, caregiver's verbal report of indoor mold could be biased and should be confirmed and quantified in future trials. Secondly, cooking indoors was related to atopic wheeze; all our families reported the use of gas-stoves that could lead to indoor pollution, as the cooking-living-sleeping spaces are often within the same area. Finally, related to pets being a risk-factor for wheezing: in our population pets are rarely cleaned, thus emanating considerable quantities of pet-allergens and house dust, the latter being a cause of allergy in almost 90% of patients with allergic rhinitis living in Villahermosa(20). A causal relationship of these facts warrants further investigated. In this context, our findings open two research questions we suggest should be tested with interventional trials: 1) does improving indoor air quality (attacking mold,

enhancing ventilation) reduce the frequency and severity of wheezing in atopic infants in tropical settings? 2) does postponing day-care attendance till > 4 months reduce the frequency and severity of wheezing in infants?.

In conclusion, infant wheezing in the tropics is frequent and most have their first episode between 2-4 months. Over forty percent of them is brought to the emergency department, conveying a considerable burden on health-care costs. Moreover, our study points at several possible risk- and protective factors related to the presence of wheezing, some of them not reported previously. Population-based studies like this one are of importance, as they could lead to hypotheses that should be tested, and at the end could lead to recommendations for public health.

Dra. Desire Larenas Linnemann

Alergóloga • Pediatra

Submission letter



Mexico City, 27th of May 2019

Dear Editor,

The here reported results form part of the "International Study of Wheezing in Infants (EISL)".

We would like to submit the manuscript reporting results of our epidemiologic study on early wheezing in atopic and non-atopic children, based on a questionnaire, validated by the international study-group. The uniqueness of our study is that it was carried out in a tropical environment, as opposed to other epidemiologic studies on early wheezing. Not even the Brazilian study related to this setting, as it was conducted near Sao Paolo with a more template climate. As such, we could detect some unique risk-factors for early wheeze, related to raising a child in a humid environment. We checked the content for completeness with the STROBE checklist for cross-sectional studies.

Also, and with more general implications, the majority of our children had their first wheezing episode between 2 to 4 months of age and in both groups, atopic and non-atopic toddlers, a common cold <3 months was a risk-factor linked to early wheeze. With the progressively better understood risk for the development of asthma of early viral infections we would like to suggest postponing day-care attendance till after 6 months. With around 40% of the wheezing leading to emergency department visits and 13% to hospitalization we showed clearly how early wheeze poses a burden on health care costs.

In conclusion, we could show risk-factors for early wheezing in atopic and non-atopic toddlers, that might lead to some generalizable suggestions focused on postponing the age of first wheezing episode and therefore reducing the burden of early wheeze and its possible consequences. Also, we could show some specific risk-factors for early wheezing in a tropical climate, not previously reported: mold indoors and gas-stove.

As Annals has been very open to the international community of investigators, both giving them free access to content and in publishing their results, and especially for the latinamerican allergists it is one of the lead journals being read, Annals was the journal we selected to submit our paper to.

Respectfully,

A handwritten signature in dark ink, appearing to read 'D. Larenas', written over a light blue circular stamp.

Désirée Larenas-Linnemann, MD, FAAAAI, Dist.Intl.FACAAI
Allergist and pediatrician

PD: According to local legislation no IRB approval nor written informed consent is needed for anonymous surveys, in which no data are revealed that could track details back to individuals. We have a letter of the Chair of the Board of Lawyers of the State of Tabasco in this respect, we could send if necessary.

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