

ENVIRONMENTAL DETERMINANTS

Environmental Risk Factors in the First Year of Life and Childhood Asthma in the Central South of Chile

ANJA BONEBERGER, M.A., M.P.H.,¹ DANIEL HAIDER,¹ JENNIFER BAER,¹ LEONIE KAUSEL, M.SC.,² RÜDIGER VON KRIES, M.D., M.SC.,³ MICHAEL KABESCH, M.D.,⁴ KATJA RADON, M.SC., PH.D.,^{1,*} AND MARIO CALVO⁵

¹*Unit for Occupational and Environmental Epidemiology, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital of Munich (LMU), Munich, Germany.*

²*Instituto de Bioquímica, Facultad de Ciencias, Universidad Austral de Chile, Valdivia, Chile.*

³*Division of Epidemiology, Institute for Social Paediatrics and Adolescent Medicine, Ludwig-Maximilians University, Munich, Germany.*

⁴*Center for Paediatrics, Clinic for Paediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Hannover, Germany.*

⁵*Instituto de Pediatría, Universidad Austral de Chile, Servicio de Pediatría, Hospital Base Valdivia, Valdivia, Chile.*

Background. Childhood asthma has a high prevalence in South America—a region of the world currently undergoing a thorough modernization and transition process. Asthma in South America is mainly associated with poor urban environment, which actually may challenge the role of the hygiene hypothesis. We systematically assessed the impact of environmental factors in the first year of life on asthma. **Methods.** A case-control study including 188 asthmatics and 294 hospital-based controls aged 6–15 years was carried out in the Central South of Chile. Parents of study participants completed a computer-assisted interview on environmental factors (such as birth order, day-care attendance, pneumonia infection, regular animal and furry pet contact, and environmental tobacco smoke exposure) in the first year of life and potential confounders. Atopy was assessed using skin prick tests. Multivariate logistic regression models were calculated to assess the association between exposures and asthma, adjusting for potential confounders. **Results.** Day-care attendance (OR = 0.31; 95% CI: 0.10, 0.94) and regular farm animal contact (OR = 0.38; 95% CI: 0.17, 0.85) were inversely related to childhood asthma in the logistic regression models. Pneumonia infection (OR = 2.24; 95% CI: 1.21, 4.16) and mold or dampness in the home (OR = 1.87; 95% CI: 1.18, 2.97) in the first year of life were positively associated with asthma. **Conclusion.** Our results suggest that the hygiene hypothesis is also applicable in the Chilean setting, a South American country in epidemiological transition.

Keywords asthma, case-control study, Chile, first year of life, infancy, hygiene hypothesis

INTRODUCTION

Asthma is the most common chronic disease in childhood worldwide. It is hypothesized that complex gene by environment interactions cause the disease. It has also been suggested that factors present around birth and in the first year of life play a prominent role for the expression of the disease (1).

According to results from International Study of Asthma and Allergies in Childhood (ISAAC), asthma in South America is characterized by large variability of disease prevalence ranging from 24.4% in Brazil to 8.4% in Mexico (2). Higher prevalence is found in underprivileged populations living in urban environments (3). In Chile, the prevalence of asthma symptoms has significantly risen in the age group 13–14 years from 10.2% in 1994 to 14.9% in 2002 (4).

In European countries, the increase of asthma has been associated with the hygiene hypothesis, which initially described the protection against asthma in children with older siblings (5) but which nowadays is understood as contact in early life with factors contributing to environments rich of microbial exposure, including family size, day-care attendance (6), and farm environments with contact to live stock (7). Pet contact in the first year of life, mainly with cats and dogs, may also have protective effects (8).

Other environmental factors in early life that may influence asthma risk are pulmonary infection (6), antibiotics (9), house dampness or mold exposure (10), and second-hand exposure to tobacco smoke (11). With respect to breastfeeding, results have been inconclusive (12).

At first glance, the hygiene hypothesis does not seem to explain the increase of asthma in South American inner cities that are characterized by poor housing, overcrowding, and unhygienic environment (3). However, studies in rural areas of South America have rarely been conducted (13). We carried out a case-control study in the Central South of Chile—a predominantly rural setting—in order to systematically investigate the

*Corresponding author: Katja Radon, Unit for Occupational and Environmental Epidemiology, Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, University Hospital of Munich (LMU), Ziemssen Street 1, 80336 Munich, Germany; Tel: +49 89 5160 2485; Fax: +49 89 5160 4954; E-mail: sekretariat-radon@med.lmu.de

association between environmental factors in the first year of life and asthma in children. We investigated birth order, day-care attendance, pneumonia, duration of breastfeeding, regular animal and furry pet contact, mold exposure, fuel used for heating, and environmental tobacco smoke exposure. The Chilean setting offers the unique opportunity to examine the validity of the hygiene hypothesis in a country of epidemiological transition (14).

MATERIAL AND METHODS

Details of the study have been described previously (15). Briefly, a hospital-based case-control study including 188 cases aged 6–15 years with asthma diagnosed by a physician and 294 controls was set up in a public hospital (Hospital Base de Valdivia) and outpatient clinic (Consultorio Externo, Avenida Francia) in Valdivia, Chile (153,000 inhabitants, 800 km south of Santiago de Chile). A “consultorio” is an institution of primary care treating publicly insured patients with general medicine and pediatrics (no emergencies). Both institutions are run by the state.

Controls with a range of diagnoses (mainly accidents, general screening exams, vaccination programs) were frequency-matched for age and sex. Controls were recruited at the same healthcare institutions as cases (Hospital Base de Valdivia and Consultorio Externo) from general medicine, internal medicine, pediatric surgery, and dentistry.

Cases and controls underwent a parental interview and skin prick test, gave blood as well as stool samples, and were visited at home where dust samples (for endotoxins, allergens) were collected. Most questions of the questionnaire were based on the internationally standardized and validated ISAAC phase II protocol (16), the Allergy and Endotoxin Study (17) and the Chronic Autoimmune Disease and Animal Contact (18) study. The questionnaire included questions about the child's experiences in the first year of life such as place of residence, number of older siblings, day-care attendance, family history of atopic diseases, and infections. The study protocol was approved by the ethics committees of the Ludwig-Maximilians University, Munich, as well as the Medical Faculty of the Universidad Austral de Chile.

Outcome and Exposure Definitions

Asthma cases were defined by a physician based on standard diagnosis criteria including lung function assessment and medical history. From 2006, when Chile adopted World Health Organization's Global Initiative for Asthma guidelines (19), diagnoses have been based on Global Initiative for Asthma criteria. For the majority of the cases, only spirometry and medical history were available, because in Chile bronchial provocation is only performed when the diagnosis is not clear after history-taking and spirometry.

Atopy was defined as sensitization to at least one of the following allergens: cat hair, dog hair, cockroach, house

dust mites (*Dermatophagoides pteronyssinus*, *Acarus siro*), mold (*Alternaria tenuis*), trees, local grass, and weed mix (Laboratorio Leti, España) (20).

Environmental factors present during the child's first year of life were derived from the computer-assisted parental interview. These early life environmental factors included place of residence (urban, i.e., living in Valdivia/rural), birth order (being first born/at least one older sibling), day-care attendance (yes/no), pneumonia infection (yes/no), duration of breastfeeding (≥ 6 months vs. < 6 months), regular (defined as at least once a week), farm animal (cattle, horses, pigs, goats, sheep, or poultry) or pet (dog, cat, or rabbit) contact, mold exposure (yes/no), fuel for heating (wood/other), and environmental tobacco smoke exposure (at least one smoker in the household/no smoker in the household).

Statistical Methods

Cross-tabulation to visualize bivariate distributions of categorical predictors and outcomes was used. Logistic regression models were carried out adjusting for the matching variables age (in quartiles: 6 to ≤ 8.3 years/8.4 to ≤ 10.6 years/10.7 to ≤ 12.8 years/ > 12.8 years) and sex (male/female). We adjusted for these variables in both bivariate and multivariate logistic regression models as suggested by Bland and Altman (21) because matching does not completely control for confounding.

Factors associated with the outcome at $p \leq .05$ were included in the final logistic regression model. These included parental atopy (if at least one parent reported “yes” on rhinoconjunctivitis or asthma) and atopic sensitization according to skin prick test. Additionally, all environmental factors considered were included in this final logistic regression model (fully adjusted model). Finally, a sensitivity analysis was conducted by restricting the final logistic model to atopic cases and all controls. Due to limited numbers, these analyses could not be repeated for nonatopic cases and controls. Because data were frequency-matched, unconditional logistic analyses could be used. All statistical analyses were carried out using SAS 9.2. (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Slightly more boys (52.66% among cases, 53.40% among controls, P^2_{χ} -test: .87) than girls took part in the study. On average, cases were 10.63 and controls 10.68 years old. Among cases, 34.04% of the parents reported having rhinoconjunctivitis or asthma, compared to 14.63% among controls (P^2_{χ} -test: $< .0001$). Allergic sensitization according to skin prick test reactivity was observed among 78.19% of cases and 46.94% of controls (P^2_{χ} -test: $< .0001$).

Table 1 shows environmental factors in the first year of life by case status. About 21.28% of the cases reported having had a pneumonia infection in the first year of life compared to 11.56% of controls. Regular contact

TABLE 1.—Prevalence of environmental factors during the first year of life by case status and bivariate results of the logistic regression models (OR $\pm$ 95% CI) adjusted for the matching variables age and sex.

Environmental factors during the first year of life	Cases (<i>n</i> = 188)		Controls (<i>n</i> = 294)		<i>P</i> -value	OR (95% CI) adjusted for age ^a and sex
	<i>n</i>	%	<i>n</i>	%		
At least one parent reporting asthma or rhinoconjunctivitis	64	34.04	43	14.63	<.0001	3.06 (1.96, 4.79)
At least one smoker in the household	104	55.32	157	53.40	.68	1.08 (0.75, 1.56)
Mold or dampness in the home ^b	82	44.09	95	32.65	.01	1.63 (1.12, 2.38)
Fuel for heating (wood/other) ^b	140	74.47	235	79.93	.15	0.73 (0.47, 1.13)
Pneumonia infection ^b	40	21.28	34	11.56	.004	2.08 (1.26, 3.44)
Day-care attendance ^b	7	3.74	19	6.48	.20	0.55 (0.23, 1.34)
Birth order (at least one older sibling) ^b	116	62.03	169	57.68	.34	0.83 (0.57, 1.21)
Breastfeeding $\geq$ 6 months	133	76.88	211	78.44	.70	0.92 (0.58, 1.46)
Living in an urban area ^c	172	91.98	268	91.16	.75	1.12 (0.57, 2.17)
Regular ^d contact with pets ^e	114	60.64	205	69.73	.04	0.67 (0.46, 0.98)
Regular ^d contact with farm animals ^f	21	11.17	54	18.37	.03	0.55 (0.32, 0.95)

Notes: ^aAge in quartiles (6 to $\leq$ 8.3 years/8.4 to $\leq$ 10.6 years/10.7 to $\leq$ 12.8 years/ $>$ 12.8 years).

^bNumbers do not add up to *N* = 482 due to missing data.

^cUrban area means Valdivia.

^d“Regular” defined as at least once a week.

^eDog, cat, rabbit.

^fCattle, horses, pigs, goats, sheep, and poultry.

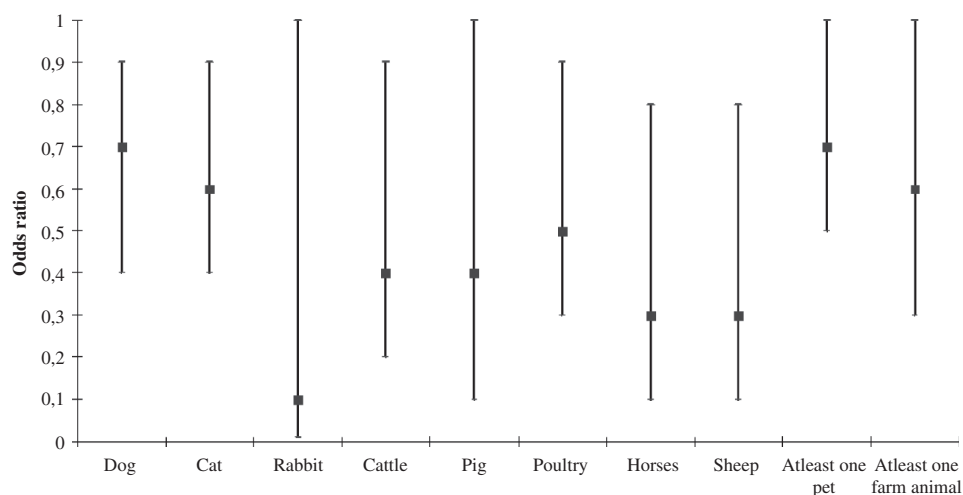


FIGURE 1.—Age- and sex-adjusted odds ratios and 95% CI comparing pet and farm animal contact during the first year of life among cases and controls (*N* = 482).

with pets was found among 60.64% of cases and 69.73% among controls. There were fewer children with regular contact to farm animals during the first year of life with a higher prevalence of farm animal contact among controls (11.17% vs. 18.37%, P^2_{χ} -test: 0.03, respectively).

In order to examine the protective effect of individual animal species, they were also analyzed separately adjusting for age and sex with OR and 95% CI (Figure 1). The inverse and statistically significant relationship between regular animal contact during the first year of life and case status was seen for all species.

In the final logistic regression model, this inverse association was confirmed for pet contact (adjusted odds ratio (aOR) = 0.70; 95% CI: 0.41, 1.04) as well as farm animal contact (aOR = 0.38; 95% CI: 0.17, 0.85).

Furthermore, asthmatics were statistically significantly less likely to attend day-care attendance in the first year of life (aOR = 0.31; 95% CI: 0.10, 0.94).

Pneumonia infection (aOR = 2.24; 95% CI: 1.21, 4.16) as well as self-reported dampness or mold exposure in the home environment (aOR = 1.87; 95% CI: 1.18, 2.97) in the first year of life was statistically significantly and positively associated with case status (Table 2).

All results were robust in the sensitivity analyses comparing atopic cases with all controls (data not shown).

DISCUSSION

In this case-control study, we investigated a broad spectrum of environmental factors in the first year of

TABLE 2.—Associations between environmental factors during the first year of life.

Environmental exposure in the first year of life	Fully adjusted ^a model (aOR)	95% CI
At least one smoker in the household	1.09	(0.70, 1.69)
Mold or dampness in the home	1.87	(1.18, 2.97)
Fuel for heating (wood/other)	0.87	(0.50, 1.50)
Pneumonia infection	2.24	(1.21, 4.16)
Day-care attendance	0.31	(0.10, 0.94)
Birth order (at least one older sibling)	0.84	(0.53, 1.33)
Breastfeeding ≥ 6 months	0.96	(0.57, 1.64)
Living in an urban area ^b	0.42	(0.16, 1.09)
Regular ^c contact with pets ^d	0.70	(0.41, 1.04)
Regular ^c contact with farm animals ^e	0.38	(0.17, 0.85)

Notes: ^aAdjusted for age, sex, parental atopy (rhinoconjunctivitis or asthma), positive skin prick test, and mutually adjusted for all other environmental exposure variables presented in the table.

^bUrban area means Valdivia.

^c"Regular" defined as at least once a week.

^dDog, cat, or rabbit.

^eCattle, horses, pigs, goats, sheep, or poultry.

life and asthma in 6–15-year-old children living in a predominantly rural area in the Central South of Chile. Overall, our findings support the validity of the hygiene hypothesis (22), which indicates that microbial exposure and farm animal and pet contact in early life may protect from asthma (23). The results also support the observation of previous studies that the protective effect of farming environment in early life is not only restricted to allergic rhinitis (7).

The hygiene hypothesis has been challenged previously with results from non-European countries, including South America, because of the high risk of asthma among inner-city urban populations from the US and South American studies (3). Such differences to our findings might be explained by the different types of exposure (e.g., farm animal contact vs. cockroaches), differences in disease phenotypes and underlying pathomechanisms, or differences in gene by environment interactions in the South American setting (7). The underlying mechanism of microbial exposure resulting in the protective effect of livestock contact on farms in early life on atopic disease is not well understood (24). Pathophysiologically, the innate immune response was shown to be involved but the exact mechanism has up to now not been unraveled. Likewise, gene by environment interactions seem to be important (6).

Whether furry pets are a risk factor (8) or a protective factor (25) for asthma remains to be debated. A recently published meta-analytic review of case-control and cohort studies concluded that cat ownership has a slightly protective effect with a relative risk (RR) of 0.7 (95% CI: 0.6, 0.9) while dog ownership slightly increased the risk for asthma (RR = 1.1; 95% CI: 1.0, 1.3) (8). Our study showed that either owning a cat or a dog in the first year of life was protective against asthma. However, almost

90% of the cat owners also owned a dog (data not shown). Therefore, we cannot disentangle the effect of dog or cat ownership in our population.

Day-care attendance in the first year of life, another factor described in the context of the hygiene hypothesis (26), was also inversely related to asthma previously. However, we could not confirm the sibling effect in our study (26). This might be due to the fact that there were few single-child families (16%) in our study population. The high number of siblings, the high prevalence of animal contact during the first year of life, and the long duration of breastfeeding indicate some of the environmental differences between South American and European or North American populations—locations, where most of the epidemiological studies on the hygiene hypothesis have so far been carried out. In this context, it might be surprising that most of our participants were considered to live in an urban area. However, urban areas in the Central South of Chile cannot be compared to inner-city urban areas in the United States or Europe. Population density and living conditions in such areas resemble more rural areas in Europe. For example, there was a considerably higher prevalence of farm animal contact during infancy in our study population than in European studies (27).

Interestingly, we also found a positive association between self-reported pulmonary infection in the first year of life and asthma. Epidemiologic results have been inconclusive with regard to the relationship of pneumonia and the development of asthma (28, 29). However, a recent study by Hilty et al. (30) assessed bacterial colonization of the lungs by 16s RNA sequencing of bacteria in bronchial lavage of asthmatic children and controls. They demonstrated that the lung—contrasting traditional knowledge—is colonized by a broad variety of bacterial species. This microbiota differs between asthmatics and nonasthmatics. It is conceivable that early pneumonia changes the lung microbiota or that early pneumonia is a first sign of these differences which, when becoming chronic, may be associated with asthma later in life.

In our study, infection was self-reported by the parents, which raises the possibility of reporting bias. As shown in a Scottish study comparing self-reported data with medical records of patient's reliability (31) for pneumonia infection was good. Therefore, we feel that the self-reported infections are a robust marker of pneumonia infection. Unfortunately, we cannot disentangle the underlying bacteria causing the pneumonia. We assessed other infections (e.g., varicella, rubella, measles) as well, but only few participants reported the occurrence of such infections during the first year of life. Therefore, the numbers were too low to analyze the associations between these infections and asthma.

Another potential risk factor in the first year of life for asthma is mold exposure (32). This was supported by our findings. However, one has to bear in mind that the validity of self-reported mold exposure might be low (33). Environmental tobacco smoke exposure in the first year of life is a commonly accepted risk factor for asthma

(11), which was not statistically significantly associated with asthma in our study. A possible explanation could be that parents of cases were more likely to underreport the exposure than parents of controls. Therefore, differential misclassification might explain this null effect. Likewise, no association between duration of exclusive breastfeeding and asthma was shown in our study. Epidemiological studies have provided controversial results showing both negative (34) and positive (35) associations between the duration of breastfeeding and asthma. One has to take into account that 90% of our study population was breastfed, with more than 75% for 6 months or more, limiting the power to show an effect.

Unfortunately, due to lack of recourses neither spirometry nor bronchial provocation could be performed in controls. However, medical history in controls was not suggestive of asthma. If there would have been a large number of controls with asthma, this would bias the results toward the null or in other words an underestimation of the effect. Therefore, our main findings are not expected to be due to bias.

In our study, 80% of our asthmatics were sensitized to at least one aeroallergen. This high prevalence of atopy among asthmatics in Valdivia confirms previous findings (36). However, risk factors for atopic and nonatopic asthma are likely to differ. Due to the low number of nonatopic asthmatics, we could not assess the association between the environmental factors under study and nonatopic asthma in our population separately. About 50% of the control group was atopic according to skin prick reactivity. This is in the range of studies among Brazilian children (37). However, it seems to be higher than in the only Chilean study including skin prick tests in the general population. In this study, only 22% of adults (mean age 25 years) were sensitized to at least one aeroallergen (38). If atopics were oversampled in our control group, this would lead to a bias toward the null resulting in an underestimation of the true effect.

This is a case-control study that can be subject to recall bias. We do assume that this recall bias is equally distributed between cases and controls and, therefore, does not lead to a differential misclassification. The face-to-face computer-assisted interviews are another possible source of bias as patients might tend to give more "desirable" answers. The interviews were conducted face-to-face and the interviewer knew about case-control status. To reduce bias, interviews were standardized and field workers were trained intensively. The results of this hospital-based study can only be generalized to other settings with caution. Patients were recruited from the general consultation; therefore, we feel that our results are representative of the general, publicly insured Chilean population (approximately 70%) (39).

CONCLUSIONS

The present analyses of a case-control study from the Central South of Chile support that the hygiene hypothesis is also valid for rural parts of Chile. Whether

this association holds true to more urban settings in South America however still needs to be confirmed.

ACKNOWLEDGMENTS

The authors are very grateful for the efforts of all field workers and technical staff (Gisela Dietrich-Gümperlein, Silvia Grau, Beate Messing, Pamela Avila, Pedro Zamorano Aguilar, Kathrin Suttner, Johanna Wagner, and Alexei Cuevas Zhabankova) for their dedication to the study. The authors thank Betty Bisdorff for reading the manuscript. Parts of this study were used for a MD and a PhD thesis. The authors are grateful to the participants for their contribution.

The research was made possible by funding by the German Research Collaboration with Developing Countries Program (DFG/BMZ), the DAAD (German Academic Exchange Service), and the German Federal Ministry of Education and Research (BMBF, International Office).

DECLARATION OF INTEREST

The authors report no competing interests. The authors alone are responsible for the content and writing of this paper.

REFERENCES

1. Turner SW, Young S, Goldblatt J, Landau LI, Le Souef PN. Childhood asthma and increased airway responsiveness: a relationship that begins in infancy. *Am J Respir Crit Care Med* 2009; 179(2):98–104.
2. Mallol J, Sole D, Asher I, Clayton T, Stein R, Soto-Quiroz M. Prevalence of asthma symptoms in Latin America: the International Study of Asthma and Allergies in Childhood (ISAAC). *Pediatr Pulmonol* 2000; 30(6):439–444.
3. Cooper PJ, Rodrigues LC, Cruz AA, Barreto ML. Asthma in Latin America: a public health challenge and research opportunity. *Allergy* 2009; 64(1):5–17.
4. Mallol J, Aguirre V, Aguilar P, Calvo M, Amarales L, Arellano P, Palma R. Changes in the prevalence of asthma in Chilean school age children between 1994 and 2002. *International Study of Asthma and Allergies in Childhood (ISAAC) – Chile phases I and III. Rev Med Chil* 2007; 135(5):580–586.
5. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299(6710):1259–1260.
6. von Mutius E. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: farm lifestyles and the hygiene hypothesis. *Clin Exp Immunol* 2010; 160(1):130–135.
7. von Mutius E, Radon K. Living on a farm: impact on asthma induction and clinical course. *Immunol Allergy Clin North Am* 2008; 28(3): 631–647, ix–x.
8. Takkouche B, Gonzalez-Barcala FJ, Etminan M, Fitzgerald M. Exposure to furry pets and the risk of asthma and allergic rhinitis: a meta-analysis. *Allergy* 2008; 63(7):857–864.
9. Foliaki S, Pearce N, Bjorksten B, Mallol J, Montefort S, von Mutius E; International Study of Asthma and Allergies in Childhood Phase III Study Group. Antibiotic use in infancy and symptoms of asthma, rhinoconjunctivitis, and eczema in children 6 and 7 years old: International Study of Asthma and Allergies in Childhood Phase III. *J Allergy Clin Immunol* 2009; 124(5):982–989.
10. Sahakian NM, Park JH, Cox-Ganser JM. Dampness and mold in the indoor environment: implications for asthma. *Immunol Allergy Clin North Am* 2008; 28(3):485–505, vii.
11. Vork KL, Broadwin RL, Blaisdell RJ. Developing asthma in childhood from exposure to secondhand tobacco smoke: insights

- from a meta-regression. *Environ Health Perspect* 2007; 115(10): 1394–1400.
12. Duncan JM, Sears MR. Breastfeeding and allergies: time for a change in paradigm? *Curr Opin Allergy Clin Immunol* 2008; 8(5): 398–405.
 13. Moncayo AL, Vaca M, Oviedo G, Erazo S, Quinzo I, Fiaccone RL, Chico ME, Barreto ML, Cooper PJ. Risk factors for atopic and non-atopic asthma in a rural area of Ecuador. *Thorax* 2010; 65(5):409–416.
 14. Luque C, Cisternas FA, Araya M. Changes in the patterns of disease after the epidemiological transition in health in Chile, 1950–2003. *Rev Med Chil* 2006; 134(6):703–712.
 15. Boneberger A, Radon K, Baer J, Kausel L, Kabesch M, Haider D, Schierl R, von Kries R, Calvo M. Asthma in changing environments – chances and challenges of international research collaborations between South America and Europe – study protocol and description of the data acquisition of a case-control-study. *BMC Pulm Med* 2010; 10:43.
 16. Weiland SK, Bjorksten B, Brunekreef B, Cookson WO, von Mutius E, Strachan DP; International Study of Asthma and Allergies in Childhood Phase II Study Group. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur Respir J* 2004; 24(3):406–412.
 17. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, Maisch S, Carr D, Gerlach F, Bufer A, Lauener RP, Schierl R, Renz H, Nowak D, von Mutius E; Allergy and Endotoxin Study Team. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347(12):869–877.
 18. Radon K, Windstetter D, Poluda AL, Mueller B, von Mutius E, Koletzko S; Chronische Autoimmunerkrankungen und Kontakt zu Tieren (Chronic Autoimmune Disease and Animal Contact) Study Group. Contact with farm animals in early life and juvenile inflammatory bowel disease: a case-control study. *Pediatrics* 2007; 120(2):354–361.
 19. Gobierno de Chile, Ministerio de Salud: Diagnóstico y Tratamiento del Asma en el menor de 16 años. Available at: www.redsalud.gov.cl/archivos/guiasges/guia_asma.pdf. Accessed April 19, 2011.
 20. Meinert R, Frischer T, Karmaus W, Kuehr J. Influence of skin prick test criteria on estimation of prevalence and incidence of allergic sensitization in children. *Allergy* 1994; 49(7):526–532.
 21. Bland JM, Altman DG. Matching. *BMJ* 1994; 309(6962):1128.
 22. Perkin MR, Strachan DP. Which aspects of the farming lifestyle explain the inverse association with childhood allergy? *J Allergy Clin Immunol* 2006; 117(6):1374–1381.
 23. Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Bohm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30(2):187–193.
 24. Kilpelainen M, Terho EO, Helenius H, Koskenvuo M. Farm environment in childhood prevents the development of allergies. *Clin Exp Allergy* 2000; 30(2):201–208.
 25. Simpson A, Custovic A. Pets and the development of allergic sensitization. *Curr Allergy Asthma Rep* 2005; 5(3):212–220.
 26. Midodzi WK, Rowe BH, Majaesic CM, Saunders LD, Senthilselvan A. Early life factors associated with incidence of physician-diagnosed asthma in preschool children: results from the Canadian Early Childhood Development cohort study. *J Asthma* 2010; 47(1):7–13.
 27. Waser M, von Mutius E, Riedler J, Nowak D, Maisch S, Carr D, Eder W, Tebow G, Schierl R, Schreuer M, Braun-Fahrlander C; ALEX Study team. Exposure to pets, and the association with hay fever, asthma, and atopic sensitization in rural children. *Allergy* 2005; 60(2): 177–184.
 28. Ramsey CD, Gold DR, Litonjua AA, Sredl DL, Ryan L, Celedon JC. Respiratory illnesses in early life and asthma and atopy in childhood. *J Allergy Clin Immunol* 2007; 119(1):150–156.
 29. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early life risk factors for current wheeze, asthma, and bronchial hyperresponsiveness at 10 years of age. *Chest* 2005; 127(2):502–508.
 30. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, Davies J, Ervine A, Poulter L, Pachter L, Moffatt MF, Cookson WO. Disordered microbial communities in asthmatic airways. *PLoS One* 2010; 5(1):e8578.
 31. Iversen L, Hannaford PC, Godden DJ, Price D. Do people self-reporting information about chronic respiratory disease have corroborative evidence in their general practice medical records? A study of intermethod reliability. *Prim Care Respir J* 2007; 16(3):162–168.
 32. Pekkanen J, Hyvarinen A, Haverinen-Shaughnessy U, Korppi M, Putus T, Nevalainen A. Moisture damage and childhood asthma: a population-based incident case-control study. *Eur Respir J* 2007; 29(3): 509–515.
 33. Douwes J, Pearce N. Invited commentary: is indoor mold exposure a risk factor for asthma? *Am J Epidemiol* 2003; 158(3):203–206.
 34. Sears MR, Greene JM, Willan AR, Taylor DR, Flannery EM, Cowan JO, Herbison GP, Poulton R. Long-term relation between breastfeeding and development of atopy and asthma in children and young adults: a longitudinal study. *Lancet* 2002; 360(9337):901–907.
 35. Fredriksson P, Jaakkola N, Jaakkola JJ. Breastfeeding and childhood asthma: a six-year population-based cohort study. *BMC Pediatr* 2007; 7:39.
 36. Calvo M, Fernandez-Caldas E, Arellano P, Marin F, Carnes J, Hormaechea A. Mite allergen exposure, sensitisation and clinical symptoms in Valdivia, Chile. *J Investig Allergol Clin Immunol* 2005; 15(3):189–196.
 37. Sarinho EC, Mariano J, Sarinho SW, Medeiros D, Rizzo JA, Almerinda RS, Sole D. Sensitisation to aeroallergens among asthmatic and non-asthmatic adolescents living in a poor region in the Northeast of Brazil. *Allergol Immunopathol (Madr)* 2009; 37(5):239–243.
 38. Rona RJ, Vargas C, Vianna EO, Bustos P, Bettiol H, Amigo H, Mackenney J, Barbieri MA. Impact of specific sensitization on asthma and rhinitis in young Brazilian and Chilean adults. *Clin Exp Allergy* 2008; 38(11):1778–1786.
 39. Unger JP, De Paepe P, Cantuarias GS, Herrera OA. Chile's neoliberal health reform: an assessment and a critique. *PLoS Med* 2008; 5(4):e79.

Copyright of Journal of Asthma is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.