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Original Article

Head: Allergic diseases and atopy among schoolchildren in eastern Croatia

Short running title: Allergy among children in eastern Croatia

Authors: Professor Neda Aberle, MD, PhD[1], Blaženka Kljaić Bukvić, MD, PhD[2], Mario Blekić, MD, PhD[2], Marko Vučković, MD[2], Darija Bardak, MD[3], Antoneta Gudelj, MD[4], Gabrijela Čančarević, MD[5], Maja Franić, MD[6]

Departments:

[1] Department of Health Studies, University of Zadar, Croatia,

[2] Department of Pediatrics, General Hospital "Dr Josip Benčević", Slavonski Brod, Croatia; Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

[3] Department of Internal Diseases, General Hospital, Požega, Croatia

[4] ENT, Head and Neck Surgery Department, General Hospital "Dr Josip Benčević", Slavonski Brod, Croatia

[5] Department of Pediatrics, General Hospital Nova Gradiška, Croatia

[6] Department of Internal Diseases, General Hospital "Dr Josip Benčević", Slavonski Brod, Croatia

Corresponding author*:* Blaženka Kljaić Bukvić MD, PhD, Department of Pediatrics, General Hospital "Dr Josip Benčević", Ulica Andrije Štampara 42, 35000 Slavonski Brod, Croatia, bkbukvic@gmail.com, mobile phone +385 98 760 766

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NA critically reviewed the manuscript and supervised the whole study process, BKB and MB adjusted the original study design to the local conditions, analysed data and write the manuscript; MV, DB, AG, GC and MF collected and anaysed data;

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ABSTRACT

Aim: A cross-sectional study was carried out in Brod-Posavina County, Croatia, to estimate prevalence of allergic diseases and atopy, as well as to investigate possible etiologic factors for asthma, allergic rhinitis and eczema in childhood.

Methods: The study included 1687 schoolchildren aged 10-11.Data were collected using standardized International Study of Asthma and Allergies in Childhood (ISAAC) Phase II written questionnaire. Skin prick tests were performed to provide an objective measure of atopy, defined as skin reactivity to one or more allergens.

Results: Lifetime prevalence for wheezing was 22.7 %, rhinitis symptoms 22.5 %, and eczema symptoms 17.9 %, respectively. Period prevalence in the past 12 months was 7.9 % for attacks of wheezing, 9.9 % for rhinoconjunctivitis symptoms, and 10.1 % for eczema symptoms. 20.2 % of the children who performed skin prick test were positive to at least one of the adopted allergens, with house dust mite sensitisation being the most frequent one. Risk factors for allergic disease include allergic disease in family, atopy, sensitisations to indoor and outdoor allergens and environmental tobacco smoke exposure at home.

Conclusion: An international comparison with results of other ISAAC Phase II studies has shown that Brod-Posavina County is an area with moderate prevalence rate for atopy and current asthma symptoms.

Keywords: prevalence, allergic diseases, atopy, children, eastern Croatia

SAŽETAK

Cilj: Istražiti prevalenciju alergijskih bolesti i atopije u dječjoj dobi na području Brodsko-posavske županije, moguće etiološke činitelje alergijskih bolesti te usporediti dobivene rezultate s rezultatima istraživača koji su koristili iste metode u Hrvatskoj i u svijetu.

Metode: Istraživanje je obuhvatilo 1687-ero školske djece dobi od 10-11 godina. Korišten je standardizirani upitnik Međunarodnog istraživanja o astmi i alergijama u dječjoj dobi za procjenu prevalencije alergijskih bolesti. Ispitanicima za koje smo dobili pisanu suglasnog roditelja, učinjen je kožni ubodni test na inhalacijske i nutritivne alergene.

Rezultati: Tijekom djetinjstva, bronhoopstrukcije je prijavilo 22,7 %, simptome rinitisa 22,5 %, a simptome atopijskog dermatitis 17,8 % ispitanika. Stopa prevalencije alergijskih bolesti u posljednjih 12 mjeseci iznosila je 7.9 % za bronhoopstrukciju, 9,9 % za simptome rinokonjunktivitisa i 10,1 % za simptome atopijskog dermatitisa. Kožni test učinjen je skupni od 1070-ero djece (63.5 %), od kojih je 20.2 % imalo pozitivan kožni test na najmanje jedan od primjenjenih alergena, s najčešćom senzibilizacijom na alergene grinje. Istraživanjem rizičnih činitelja, uočeno je kako alergijske bolesti u obitelji, alergijska senzibilizacija i izloženost duhanskom dimu povećavaju rizik za alergijske bolesti u dječjoj dobi.

Zaključak: Usporedbom s drugim međunarodnim centrima, Brodsko-posavska županija se svrstava u područja s umjerenom prevalencijom alergijskih bolesti i atopije u dječjoj dobi.

Ključne riječi: prevalencija, alergijske bolesti, atopija, djeca, istočna Hrvatska

BACKGROUND

Allergic diseases with a complex aetiology like asthma, allergic rhinitis and eczema are the most common chronic conditions in childhood. Prevalence of these allergic diseases has constantly and significantly increased worldwide over the recent decades.[1]Over the last several years, countries in transition have experienced a dramatic increase in allergic diseases.[2] However, publications from high-income countries at the end of the 20th century reported a moderate decrease in asthma and atopic diseases in older age groups of children.[3-6] Moreover, according to the data from ISAAC Phase III, previous observations that asthma is more prevalent in affluent than in non-affluent countries,[7] cannot be considered accurate. This epidemiological survey has documented great variability in asthma and allergic disease prevalence not just between different countries, but also between centres in the same country. The cause of recorded increase and differences in prevalence is complex. It has been assumed that the changes in local environmental exposures, combined with genetic susceptibility could account for the observed variability. The observed environmental risk factors for allergic diseases include indoor and outdoor environment, diet, lifestyle, stress, use of antibiotics and family size. Complete development of allergic phenotype is influenced by the period in which it has occurred (early childhood - prenatal and early postnatal). However, epidemiologic data indicate differences in risk factors for asthma between countries.[8]

Although Croatian researchers, (a country that is part of both Mediterranean and Central Europe)[9-16] have previously written about asthma, rhinitis and eczema in various parts of Croatia, there was little data about atopy, environmental exposures and life style which can be associated with asthma among the Croatian population. Considering the great variability of published data in terms of prevalence of allergic

diseases between different centres in Croatia and little published data of predisposing exposures, the aim of our study was to research prevalence of asthma, allergic rhinitis, eczema and atopy in childhood in Brod-Posavina County (Eastern Croatia), as well as the risk factors for these allergic diseases.

METHODS

Study design and setting

Cross-sectional study was carried out in Brod-Posavina County, which is part of Slavonia, one of the four historical regions of Croatia. It is located in the Pannonian Basin, which is bordered by the Danube, Drava and Sava rivers. The western part of the region consists of the Sava and Drava vallies, and the mountains and hills surrounding the Pozega valley. The tallest among them is 984-metre tall Psunj. The eastern part of the region consists of plains. Slavonia enjoys a moderately warm and rainy continental climate, with relatively low precipitation. Parental consent was obtained from parents before the study was carried out. The study was approved by the ethics committees of the participating institutions.

Participants

Between the September 2007 and June 2008, children aged 10-11 from all elementary schools (N=2224) in the Brod-Posavina County were asked to participate in the study, which included a questionnaire for parents and a skin prick test.

Methods

The data were collected using standardized International Study of Asthma and Allergies in Childhood (ISAAC) Phase II written questionnaire.[17-19]

The *core questionnaire* includes demographic characteristics: gender of child, parental age and education, education of mother and father and family history of atopy and the questionnaires on wheezing, rhinitis and eczema.

Supplementary questionnaires includes the risk factor questionnaire which inquiring about early days: birth weight, breast feeding, attendance of nursery or kindergarten and number of older siblings. The second part of the questionnaire refers to the characteristics of the child's home during the conducting the survey and during the first year of life: pet ownership, farm animals contact, cooking/heating fuell, surroundings and environmental tobacco smoke exposure.[19]

Skin prick test (SPT) was performed to provide an objective measure of atopy, defined as skin reactivity to one or more allergens. Sensitisation to eight allergens was assessed: *Dermatophagoides pteronyssinus*, dogs, cats, eggs, peanut, ragweed, birch and mixed grass pollen. Standardized allergen extracts and control solution were obtained from Stallergens (France). A weal size ≥3mm after subtraction of negative control was considered positive. The presence of visible flexural dermatitis was determined through skin examination.

Statistical Analysis

Statistical analysis was carried out using SPSS 19 (SPSS Inc., Chicago, IN, USA). Symptom prevalences were calculated by dividing the number of positive responses to each question by the number of completed questionnaires. We analysed associations between environmental exposures and main research outcomes (current wheezing, current rhinoconjunctivitis and current eczema) using logistic regression. Results are presented as p values, odds ratios (OR) and 95% confidence intervals (95%CI) as a measure of effect size.

RESULTS

Of 2224 children who were approached, 1687 agreed to participate in the study (response rate 75 %; 847 (50.2 %) males and 840 (49.9 %) females). Descriptions of the population are shown in Table 1. We observed that 67.7 % children had mothers under the age of 40, and 56 % of them had father above the age of 40. Parents had elementary or high school education (only 9.4 % of children have a mother and 11.6 % have a father with academic education). Positive family history of atopy has 1/5 of participans. We observed that 2/3 of the children who participated grew up in small families (one or two children).

Of the families that participated in the research, 1/5 own a pet (a cat or a dog), but only 14 % of them had pets (a cat or a dog) while their child was an infant. Current contacts with farm animals were reported for 16.9 % children, while 9.6 % children were in contact with farm animals in infancy. Families mostly used gas for cooking and wood as the heating fuel. In rural areas live 59.5 % children, while 40.5 % come from urban surrounding, Table 2. At home, 54.1 % children were currently exposed to environmental tobacco smoke (ETS) by any household member, Table 3.

Prevalence

Prevalence of allergic disorders is shown in Table 4. According to written questionnaire, wheezing during childhood was observed in 383 (22.7 %) children. Wheezing during previous 12 months reported 134 (7.9 %) children, with significantly more wheezing in boys than girls (59.7 % *vs*. 40.3 %, p=0.02). Prevalence rate for doctor diagnosed asthma was 4.1 %, with significantly more boys than girls suffering from asthma (boys 63.2 % *vs*. girls 36.8 %, p=0.03).

Lifetime prevalence of rhinitis symptoms was 22.5 %, while 325 children (19.3 %) reported having the same nasal problems during past 12 months. In 167 cases (9.9 %), nasal symptoms were associated with conjunctivitis. There was no gender difference in nasal symptoms. Itchy rash was reported by 301 (17.9 %) of children, with lower prevalence of eczema symptoms during the previous 12 months (10.1 %). The total of 1.9 % of examined children had a visible flexular dermatitis.

The participation rate for SPT was 48.1 % (1070/2224). The prevalence rate of atopy was 20.2 % (216 children had a positive skin prick test to at least one of the adopted allergens), with the house dust mite sensitisation being the most frequent one (146/1070, 13.6 %). The prevalence of atopic wheeze (defined as current wheeze plus skin prick test reactivity) was 3 %.

Risk factors for current wheezing

The risk factors for current wheezing were: male gender, environmental tobacco smoke exposure now and during early life, family history of atopy, atopy, positive SPT to *D. pteronyssinus*, grass and ragweed. (Table 6). Symptoms of rhinoconjunctivitis and eczema were risk factors for wheezing over the last 12 months. Parental education, place of birth and place of life, events in perinatal and infant period of life (weight at birth, term of birth, breast-feeding, attending a child care or kindergarten), home environment and surrounding area (urban/rural; contact with furry pets and farm animals; damp spots, moulds or fungus on the walls; fuel used for cooking and heating, were not associated with asthma).

Risk factors for current rhinoconjunctivitis

We observed significant connection between the current rhinoconjunctivitis and family history of allergic disorders and atopy. Positive SPT to *D. pteronyssinus*,

ragweed and birch increased, while the influence of rural surrounding in the 1st year of life reduced the risk for current rhinoconjunctivitis. Wheezing and eczema were strongly associated with current nasal and eye symptoms, Table 6.

Risk factors for current eczema

Current eczema was significantly higher among girls compared to boys. Family history of atopy increased the risk for eczema, which was even greater if the mother had also suffered from an allergic disease. We observed significant associations between current eczema and positive SPT *D. pteronyssinus*, wheezing and rhinitis, Table 6.

DISCUSSION

The results of our study (within the context of ISAAC Phase II) contribute to previously published data on the prevalence of asthma and allergic disease in childhood. Lifetime prevalence for wheezing, rhinitis symptoms and eczema symptoms was 22.7 %, 22.5 %, and 17.8 %, respectively. Period prevalence in the last 12 months was 7.9 % for attacks of wheezing or whistling in the chest, 19.3 % for rhinitis, 9.9 % for rhinoconjunctivitis symptoms, and 10.0 % for eczema symptoms. In addition, we first researched the prevalence of atopic sensitisation and the risk factors for the symptoms of allergic disease in Croatian schoolchildren. Among skin prick tested children, 216 (20.2 %) had positive reaction to at least one of the adopted allergens, with house dust mite sensitisation being the most frequent one. The percentage of positive skin prick test for each allergen was *D. pteronyssinus* 13.6 %, ragweed 7.3 %, grasses 4.9 %, dog 2.6 %, birch 2.2 %, egg 1.8 %, cat 1.6 %, and peanut 1.6 %. The strongest *risk factors for current wheezing* were specific sensitisation to *D. pteronyssinus* and current eczema. Also, positive SPT to ragweed

increased the risk for asthma. Sensitisation to grass and atopy contributed to fourfold increasing in the risk for current wheezing. ETS, especially during infancy, family history of atopy, rhinitis, current rhinoconjunctivitis, and male gender were independent risk factors for wheezing in the past 12 months.

Positive SPT to pollen (ragweed and birch) was the strongest *risk factor for current rhinoconjunctivitis*. Also, family history of allergic disorders, atopy, positive SPT to *D.pteronyssinus,* wheezing (current and ever) and eczema increased the risk, while rural surrounding in infancy reduced the risk for current nasal and eye symptoms.

The strongest *risk factors for current eczema* include allergic diseases: rhinitis and wheezing. We also observed the increased risk among girls, children with family history of atopy and specific sensitisation to *D. pteronyssinus.*

The design of our study was cross sectional. We followed up methodology of ISAAC Phase II using standardized written questionnaire of self-reported symptoms. Recommended sample size was 3 000 to ensure good prevalence estimates for severe asthma, but centres like the one in Brod – Posavina County, with small number of inhabitants, were also included in ISAAC. We did not use a video questionnaire. The data of environmental exposures, like ETS exposure, was assessed by a questionnaire rather than objective measurement (e.g. cotinine) and collected retrospectively, which may affect the real exposure. Also, environmental exposures may be influenced by behaviour and affection status of parents towards the children who participated.

The prevalence of asthma and allergic disease dramatically increased in the second half of the 20th century. The data which show the increase in asthma prevalence among Croatian children was collected in a similar manner as the data

from the whole world in the same period of time (using surveys with non-standardized methodologies).[11] Over the last two decades, in different Croatian counties, several standardised epidemiological surveys (using written and video questionnaire ISAAC Phase I and written questionnaire Phase II) were conducted.[11-13, 15] They included different age groups of children: 6-7 years, 10-11 years, 12-14 and 14-16 years. In comparison to research conducted in Zagreb[15], Primorsko-goranska County[11] and Medjimurje County[13], we observed higher prevalence rate for current wheezing (Brod-Posavina County 7.9 %) in relation to Zagreb (6.02 %) and Medjimurje County (5.11 %), but lower than in Primorsko-goranska County (age group 6-7 years: 9.7 %; age group 13-14: 8.4 %) and Splitsko-dalmatinska County (9.7 %).[12] Prevalence rate of rhinoconjunctivitis was higher in Zagreb (12.13 %), than in this (Brodsko-posavska County 9.9 %) and other Croatian studies (Medjimurje County 7.14 %, Primorsko-goranska County – age group 6-7 years 5.6 %, age group 13-14 years 6.7 %). Eczema was most prevalent in Brod-Posavina County 10.1 %, compared to three other studies (Zagreb 6.1 %, Medjimurje County 5.34 %, Primorsko-goranska County (age group 6-7 years: 6.1 %; age group 13-14: 4.8 %). According to the results of previously reported Croatian studies, the authors observed different prevalence rates for asthma and allergic disease in various regions. That could have been influenced by different geographic location, climate, diet, vegetation and socio-economical factors. The reports of ISAAC showed a wide variability in the prevalence of allergic diseases which occur not just between regions but also among centres of the same county. Also, prevalence rates did not correlate with the economic status, altitude and air pollution. The observed great variability has also suggested an important role of individual exposures and life style as well as genetic background.[20] According to the data from different parts of Croatia, moderate prevalence rates of asthma,

rhinoconjunctivitis and eczema were observed among Croatian schoolchildren in the period from 2005 to 2011, while a recent study has shown a further rising trend.[10]

An atopic sensitization has long been known to be related to childhood asthma.[21] ISAAC Phase II has researched the prevalence of atopic sensitization and its role in asthma. 54 439 children from 22 countries were included, and 31 759 were skin prick tested. The prevalence of skin prick test reactivity was lowest in Kintampo, rural Ghana (1.7 %), and highest in Hong Kong, China (45.3 %). There were no correlations between prevalence rates of current wheeze and atopic sensitization with the gross national income (GNI). However, researchers observed that associations between atopic sensitization and asthma symptoms increased with economic development.[1] Prevalence rate of atopy was 20.2 %. Positive skin prick test to at least one of the adopted allergens was a strong risk factor for current wheeze and symptoms of rhinoconjunctivitis. Croatia, located in Central Europe, had GNI of 19,731 US$ per capita. Prevalence rate of atopy and associations with asthma were in correlation with prevalence of atopy among countries with similar GNI *per capita,* like Estonia, Turkey, and Latvia.[1]

Novel investigations have shown that relationship between allergen exposure, atopy and asthma is not clear. Atopy and asthma are not single phenotypes. Allergen exposures in interaction with other environmental exposures had different outcomes on atopy and asthma phenotypes; depending on individual genetic background.[22] We have observed the importance of asthma heredity in our research. Family history of atopy, especially atopic mothers increased the risk for asthma in their offspring. Asthma was more common among boys than girls, which might be explained by anatomical differences of lungs in preschool period. The environmental exposures like allergens and specific sensitization may play an important role in particular asthma phenotypes.[23] Skin test reactivity to indoor (*D.pteronyssinus*) and outdoor allergens (grasses, ragweed) has increased the risk for asthma in our study group. Tobacco smoke is the most prevalent indoor pollutant. It is estimated that half the children worldwide are exposed to second-hand smoke. *In utero* and childhood exposure to environmental tobacco smoke is associated with an impaired lung function and constitutes a risk factor for development of asthma.[24] In our study population, tobacco smoke exposure by the mother was observed for 19.3 % of the children *in utero* and 30.7 % of the children during the 1st year of life. According to different cross sectional studies, prevalence of mothers who smoke during pregnancy differs between populations (4.4 %-28.7 %).[25] Children exposed to ETS in infancy had two-fold increase in the risk for asthma in current research. Our results are consistent with the observation that ETS exposure in early life by the mother (mother smoking during pregnancy and the 1st year of life) is associated with impaired lung function and constitutes a risk factor for development of asthma.[24]

Modern diet characterized by an increased consumption of meat and fast food has been suggested to be the risk factor for asthma.[26] However, we have found no significant associations between diet and asthma symptoms (low frequency of fast food consumption in our study population). *Rhinoconjunctivitis* is strongly associated with family history of allergic disease, whereas sensitisation to *D. pteronyssinus,* ragweed and birch increased the risk for nasal symptoms. In addition, we have observed a protective effect of rural surrounding in early life on current rhinoconjunctivitis symptoms. Previous studies have concluded that growing up in a rural environment (through contact with farm animals, stables, endotoxin, and consumption of unpasteurized milk, more pets and siblings) reduced prevalence of atopy, hayfever and asthma.[27, 28]

Interaction between susceptibility genes, environment and immunological factors contributes to the pathogenesis of eczema. Heredity is complex and includes mutations in a number of genes (genes of skin barrier and genes of innate and adaptive immunity). In this research, family history of atopic disease and female gender has increased the risk for eczema. Croatian authors from Medjimurje have analysed the risk factors for the development of atopic dermatitis in children. Their results, as well as the results from other epidemiological studies, are consistent with our conclusion.[14] Mutation in skin barrier genes impairs skin integrity and reduces the resistance to external influences. Damaged skin facilitates penetration of allergens and contributes to the development of sensitization to aeroallergens. This might explain higher proportion of positive SPT to *D. pteronyssinus* among children with eczema in our research.

The symptoms of rhinitis and wheezing ever during childhood and during research have increased the risk for atopic dermatitis in the current study. This is consistent with majority of epidemiological studies on a population level, which link allergic disease through “atopic march”.[29, 30] However, research on heterogeneity of allergic disease on an individual level, has shown that co-occurrence of eczema, rhinitis and asthma are the characteristics of only one distinct phenotype.[31]

Conclusion: We have observed a moderate prevalence of allergic disease and atopy among Croatian schoolchildren. The risk factors for allergic disease in our study population include allergic disease in family, atopy, sensitisations to indoor and outdoor allergens and environmental tobacco smoke exposure. The findings from this epidemiological survey provide the basis for further studies, which would bring better understanding of factors associated with allergic disease in childhood and provide novel public health intervention to reduce prevalence of asthma.

Table 1. Characteristics of study population

Characteristic	n*	n(%)
mother age	1687	
below 40		1152 (67.7 %)
above 40		535 (31.4 %)
father age	1687	
below 40		734 (43.1 %)
above 40		953 (56 %)
mother education/year	1654	
0-12 years		1494 (87.8 %)
13 or more years		160 (9.4 %)
father education/year	1635	
0-12 years		1435 (84.3 %)
13 or more years		200 (11.6 %)
family history of atopy	943	363 (21.3 %)
birth weight	1651	
<1999g		51 (3 %)
2000-2499g		96 (5.6 %)
>2500g		1504 (88. 4%)
breast feeding-yes	1687	1535 (90.9 %)
nursery	1662	225 (13.2 %)
kindergarten	1663	499 (29.2 %)
older siblings	1687	
0		565 (33.2 %)
1		547 (32.1 %)
2 or more		575 (33.8 %)

*n-number of participants with valid answer

Table 2. Characteristic of child household during conducting the questionnaire and during the 1st year of life

Characteristic	n*	during conducting the questionnaire	n*	during the 1st year of life
cat in home	1611	360 (21.2 %)	1617	248 (14.6 %)
dog in home	1619	374 (22.0 %)	1626	245(14.4 %)
contact with farm animals	1626	287 (16.9 %)	1620	164 (9.6 %)
cooking fuell				
electricity	1650	642 (37.7 %)	1636	627 (36.8 %)
gas	1644	1404(82.5 %)	1638	1207 (70.9 %)
wood or coal	1646	561 (33 %)	1636	699 (41.1 %)
heating fuell				
electricity	1634	150 (8.8 %)	1629	177 (10.4 %)
gas	1633	554 (32.6 %)	1626	285 (16.8 %)
wood	1651	1114 (65.5 %)	1638	1222 (71.8 %)
coal	1634	21 (1.2 %)	1624	28 (1.7 %)
oil	1622	56 (3.3 %)	1611	122 (7.2 %)
surrounding area	1675		1675	
rural		996 (59.5 %)		991 (58.7 %)
urban		679 (40.5 %)		677 (40.1 %)

*n- number of participants with valid answer

Table 3. Environmental tobacco smoke exposure by mother and any household member through three stages of child life (during conducting the questionnarie, 1st year of life, pregnancy)

	n*	during conducting the questionnarie	n*	during 1st year of of life	n*	during pregnancy
mother	1663	613 (36.0 %)	1656	523 (30.7 %)	1646	328 (19.3 %)
any household member	1660	921 (54.1 %)				
number of cigarets						
<10	929	374 (22.0 %)				
10-20	929	406 (23.9 %)				
>20	929	149 (8.8 %)				

*n- number of participants with valid answer

Table 4. Prevalence of wheezing, rhinitis, rhinoconjunctivitis and eczema

Simptoms of allergic disease	N sample	positive answer	Prevalence
wheezing ever	1684	383	22.7 %
wheezing in the last 12 months	1684	134	**7.9 %**
dg asthma	1672	68	4.1 %
rhinitis ever	1683	379	22.5 %
rhinitis in the last 12 months	1687	325	**19.3 %**
rhinoconjunctivitis in the last 12 months	1683	167	**9.9 %**
dg pollinosis	1678	106	6.3 %
eczema ever	1680	301	17.9 %
eczema in the last 12 months	1680	170	**10.1 %**
dg eczema	1676	198	11.8 %

Table 5. Prevalence of atopic sensitisation

	N sample	positive SPT*	%
atopy	1070	216	20.2 %
D.pteronyssinus	1070	146	13.6 %
cat	1070	17	1.6 %
dog	1070	28	2.6 %
birch	1070	24	2.2 %
ragweed	1070	78	7.3 %
grasses	1070	52	4.9 %
egg	1070	19	1.8 %
peanut	1070	17	1.6 %

*SPT- skin prick test

Table 6. Risk factors for allergic disease: wheezing during last 12 months (i.e. current wheezing), rhinoconjunctivitis during last 12 months (i.e. current rhinoconjunctivitis), eczema during last 12 months (i.e. current eczema)

Risk factor	Current wheezing			Current rhinoconjunctivitis			Current eczema		
	OR[¤]	95%CI[¥]	p[†]	OR	95%CI	p	OR	95%CI	p
male gender	1.51	1.06-2.15	0.03	NS	NS	NS	NS	NS	NS
female gender	NS[§]	NS	NS	NS	NS	NS	1.39	1.01-1.91	0.02
mother smoking current	1.65	1.43-2.4	0.01	NS	NS	NS	NS	NS	NS
1st year	2.2	1.5-3.23	0.00	NS	NS	NS	NS	NS	NS
family history of allergic disease	2.26	1.42-3.6	0.00	2.87	1.76-4.67	0.00	2.32	1.56-3.45	0.00
rural surrounding - 1st year	NS	NS	NS	0.61	0.4-0.93	0.02	NS	NS	NS
Atopy	4.03	2.27-7.15	0.00	2.15	1.28-3.6	0.00	NS	NS	NS
SPT[‡‡] *D.pteronyssinus*	7.65	3.91-14.97	0.00	1.99	1.12-3.54	0.02	1.82	1.07-3.1	0.03
SPT grasses	4.74	1.62-13.85	0.01	3.32	1.09-10.15	0.03	NS	NS	NS
SPT ragweed	5.47	2.26-13.27	0.00	3.03	1.59-5.78	0.00	NS	NS	NS
wheezing ever	NA[§§]	NA	NA	2.95	1.96-4.44	0.00	3.93	2.69-5.74	0.00
current	NA	NA	NA	2.54	1.45-4.47	0.00	4.17	2.24-7.78	0.00
rhinitis ever	17.67	11.34-27.55	0.00	NA	NA	NA	6.95	4.75-10.15	0.00
current	15.97	10.21-24.97	0.00	NA	NA	NA	9.38	6.27-14.01	0.00
eczema ever	5.52	3.48-8.74	0.00	1.89	1.24-2.88	0.00	NA	NA	NA
current	7.47	4.16-13.43	0.00	2.67	1.48-4.79	0.00	NA	NA	NA

[¤] OR-Odds Ratio;

[¥] 95%CI-95% Confidence Interval;

[†] $p<0.05$;

[‡‡] SPT - Skin PrickTest;

[§] NS - non significant;

[§§] NA- non aplicable analysis

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