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ENVIRONMENTAL AND LIFESTYLE FACTORS,
INCLUDING VIRAL INFECTIONS, IN RELATION TO
DEVELOPMENT OF ALLERGY AMONG CHILDREN
IN SAINT-PETERSBURG AND STOCKHOLM

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ENVIRONMENTAL AND LIFESTYLE FACTORS

Anna Sidorchuk

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**Karolinska
Institutet**



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Institutet**

From The Institute of Environmental Medicine
Karolinska Institutet, Stockholm, Sweden

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***To my mother
and Gudmundur***

***Ars longa, vita brevis, occasio praeceps,
experimentum periculosum, iudicium difficile.***

Hippocrates, ancient Greek physician
(ca 460 BC – ca 370 BC)

ABSTRACT

The increasing prevalence of allergic diseases in children, particularly noteworthy in developed countries, has become an object of international concern. A lack of exposure to a broad range of infectious agents in early life has been suggested as one of the contributing factors. An increase in allergy prevalence in Eastern Europe, including Russia, similar to that earlier noted in Western countries has recently been shown, suggesting that “Westernization” may contribute to the geographical differences. However, information based on validated, population-based studies on allergic diseases in Russian children is limited and the role of various environmental as well as lifestyle factors has not been clarified.

In the first two studies the role of Epstein-Barr virus (EBV) and Cytomegalovirus (CMV) infections was investigated, including a potential interaction between the two viruses, for development of allergic conditions and sensitization in Swedish 4 year-old children. From a Swedish prospective birth cohort study on factors of importance for development of allergy, 2,581 children were enrolled. The classification of allergic diseases was based on questionnaire answers and determination of IgE-antibodies to common airborne and food allergens. Immunoglobulin G (IgG) to EBV was determined by indirect immunofluorescence and to CMV by an enzyme-linked immunosorbent assay. Total seropositivity to EBV and CMV was found in 53% and 46% of the children, respectively, while joint seropositivity was detected in 25%. There were no significant associations between seropositivity to either EBV or CMV and allergic manifestations. Seropositivity to CMV alone was related to IgE antibodies to airborne and food allergens. An antagonism between CMV and EBV in relation to sensitization to airborne and food allergens was suggested.

Two other studies were aimed at providing internationally comparable data on the prevalence of allergic diseases and sensitization among Russian children as well as at elucidating the role of various environmental and lifestyle factors, including farm-related exposures. The study population comprised 1,702 children aged 2-7 years from Saint-Petersburg. A cross-sectional questionnaire-based survey was performed focusing on environmental and lifestyle factors as well as on occurrence of allergic diseases. Blood sampling was performed in a subgroup to determine allergen-specific IgE-antibodies to the most common airborne and food allergens. The response rate to the questionnaire survey was 85.1%. Allergic diseases were reported for 23% of the children and more than 30% exhibited IgE-antibodies to common allergens. Among environmental factors, physician-diagnosed asthma was associated with exposure to environmental tobacco smoke and wood smoke. Similar relations were indicated for current asthma and rhinitis symptoms as well as for diagnosed allergic rhinitis, but not for sensitization. Prenatal exposure to certain livestock, such as pigs, was associated with an increased prevalence of allergic diseases, but not with sensitization. Regular consumption of farm milk tended to decrease the risk of allergic conditions.

In conclusion, the studies do not support the hypothesis that EBV or CMV infections in early childhood influence the pathogenesis of allergic diseases or allergen specific IgE-sensitization in children at 4 years of age. However, an EBV/CMV antagonism was

suggested with respect to sensitization, underlining the importance of studying of viral interactions. The occurrence of allergic diseases and sensitization among children from Saint-Petersburg appears similar to the prevalence in Northern and Western Europe as well as the panorama of risk factors.

Key words: *allergic diseases, children, Cytomegalovirus, environmental tobacco smoke, Epstein-Barr virus, farm-related exposure, sensitization, wood smoke*

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

Adj	Adjusted
CI	Confidence interval
CMV	Cytomegalovirus
EBV	Epstein-Barr virus
ETS	Environmental tobacco smoke
Fx5[®]	A mix of common food allergens: cow's milk, hen's egg, wheat flour, peanut, soy bean and codfish
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IIF	Indirect immunofluorescence
IL	Interleukin
ISAAC	The International Study of Asthma and Allergies in Childhood
OR	Odd ratio
Phadiatop[®]	A mix of common inhalant allergens: cat, dog, <i>Dermatophagoides pteronyssinus</i> , horse, birch, mugwort, timothy, <i>Cladosporium herbarum</i>
TNF	Tumor necrosis factor
Th	T helper
VCA	Virus capsid antigen

1 INTRODUCTION

By the end of 20th century, descriptive data on asthma and allergic diseases indicated a substantial and persistent increase in prevalence. The increase appeared particularly strong in developed countries, especially among children [1-3]. However, in recent years several epidemiological surveys revealed a tendency to stabilization in allergy prevalence [4-7]. These trends in prevalence seen worldwide over the past several decades, can not be explained by genetics and may reflect changes in diagnostic practice, medical care, lifestyle or exposure to environmental factors [3, 4, 8-10].

It has been postulated that among nongenetic risk factors, a complicated interplay between infections, occurring particularly early in life, and the immune system could affect the development of allergic diseases [11-17]. A variety of infectious agents have been of scientific interest, however, the effect of Herpesvirus infections on allergic conditions has been studied to only limited extent [18-22]. Among them, Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) often occur early in life and partly favor antagonistic immune responses, which may counteract the immunological effect of each other. One of the purposes of this thesis was to elucidate the role of CMV and EBV infections, including a possible interaction between these two viruses, for the development of allergic diseases and sensitization in children.

There are substantial international differences in the prevalence of allergic conditions, particularly between the industrialized world and developing countries [1, 23-26]. Despite the interest in West-East comparisons of the pattern of allergic morbidity, epidemiological data on allergy across Western European countries have been obtained to a much larger extent than from Central and Eastern Europe [1, 11, 14, 23-38]. Validated and population-based studies on the prevalence and development of allergic diseases in Russian children are very few [23, 32-36, 39-42], therefore, the role of various environmental and lifestyle exposures has not been clarified. Another purpose of the thesis was to provide internationally comparable data on the prevalence of asthma, allergic rhinitis, eczema and sensitization among urban Russian children of pre-school age as well as to assess the associations between various environmental exposures or lifestyle factors and allergic diseases in early childhood.

2 BACKGROUND

2.1 DEFINITIONS OF ALLERGIC DISEASES

The term "allergy" from the Greek *allos* ('other') and *ergon* ('work') was introduced in 1906 in *Munchener Medizinische Wochenschrift* by von Pirquet, who recognized that in both protective immunity and hypersensitivity reactions, an external agent had induced some form of "changed or altered reactivity" [43, 44]. The term "atopy" (from the Greek *atopos*, meaning "out of place") is often used to describe immunoglobulin E (IgE) mediated diseases [44], i.e. a hereditary predisposition to produce IgE-antibodies against common environmental allergens or to develop adverse immune reactions involving IgE-antibodies [44]. A schematic overview of nomenclature and classification of allergic diseases, as proposed by Johansson, et al. [45], is presented in Figure 1.

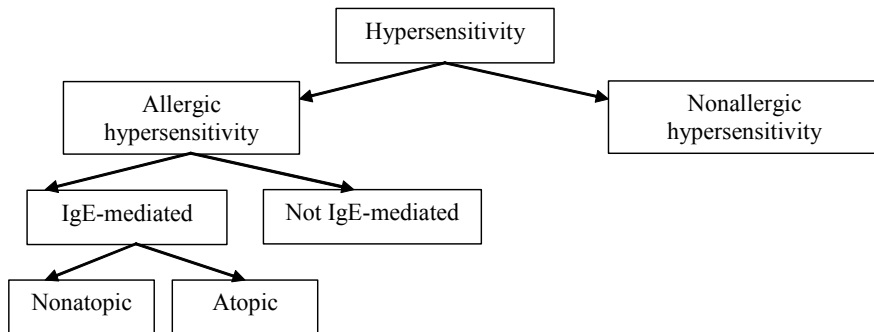


Figure 1. A schematic nomenclature for allergic diseases. (Source: Johansson, et al, 2001 [45])

Typical allergic symptoms include asthma, rhinoconjunctivitis, gastrointestinal symptoms, and characteristic skin lesions, generally referred to as "atopic diseases" [45]. Asthma (from the Greek word meaning "breathless") has been defined as follows: "*Asthma* is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment" [46]. Allergic rhinitis and atopic dermatitis have been defined as follows: "*Allergic rhinitis* is an immunologically mediated hypersensitivity reaction of the nasal passages, usually characterized by episodes of sneezing, itching, rhinorrhea, and nasal obstruction" [45, 47]; "*Atopic dermatitis* is eczematous hypersensitivity reactions in the skin characterized by an itchy red rash, consisting of tiny

papules, sometimes with an urticarial component, which may form confluent red sheets” [45, 47].

One of the main concerns of epidemiological studies on allergic diseases in children is to use standardized methodology to define the disease. For childhood *bronchial asthma* the following clinical definition could be used: “three or more episodes of wheezing before 2 years of life, or one from 2 years of age, or any episodes of wheezing independent of age, if combined with asthma in the family or other atopic symptoms in the child”. In some studies, the diagnosis “asthma” is used if two or more episodes of obstructive bronchitis occurred. There are no widely agreed criteria for the diagnosis or classification of *allergic rhinitis*, and it may vary partly depending on age group under study [48]. It has been defined as rhinitis or rhinoconjunctivitis if symptoms appear at least twice after exposure to a particular allergen unrelated to infection. For the clinical and epidemiological determination of *atopic dermatitis* a list of major and minor criteria proposed by Hanifin and Rajka [49-51] are applicable.

In Russian allergological practice the approach to define allergic diseases in children is rather similar to the above mentioned, particularly with respect to allergic rhinitis [52] and atopic dermatitis [53]. However, it is not very common to diagnose asthma based on a history of episodes of obstructive bronchitis and this may, to some extent, contribute to the differences in prevalence of this disease seen in various studies.

2.2 INTERNATIONAL EPIDEMIOLOGICAL PATTERNS OF ALLERGY

2.2.1 Importance of international comparisons

Many of the epidemiological hypotheses, particularly on causality of chronic diseases, have their origins in international comparisons [54-56]. The systematic investigation of patterns of disease prevalence and incidence across geographical areas, demographic groups and time have generated many hypotheses on etiology in the fields of cancer and cardiovascular diseases [57-60]. Allergic conditions show substantial international differences in disease prevalence, particularly between the industrialized world and developing countries [1, 23]. It is clear that the possible causes of etiologically multifaceted diseases, such as allergic conditions, can not be revealed from laboratory analyses or clinical case reports only. Important associations with lifestyle factors and environmental factors may become apparent when comparisons are made between populations exposed to risk factors in sufficiently different manner [61]. Some recent achievements in epidemiology of childhood allergic disorders have been made in international and multiregional studies, in which major inter-country differences in prevalence and trends of allergic diseases were established. Thus, the ISAAC study (The International Study of Asthma and Allergies in Childhood) performed in 56 countries across the world revealed more than 20-fold differences in prevalence of allergic symptoms in children from different countries [1, 23, 48, 62]. In the international PARSIFAL (Prevention of Allergy Risk Factors for

Sensitization In Children Related to Farming and Anthroposophic Lifestyle) [9, 63-65] and ALEX (Allergy and Endotoxin study) [26] studies growing up on a farm and having an anthroposophic lifestyle appeared to be protective for both sensitization and allergic diseases in childhood. In the multicentre CESAR study (Central European Study of Air Pollution and Respiratory Health) [25] a higher prevalence of bronchitis in Central and Eastern European countries was observed than in Western Europe, while prevalence of asthma appeared lower than in Western Europe, however, the differences in prevalence could partly be attributable to diagnostic practice. The Italian multicenter study SIDRA (The Italian studies on Respiratory Disorders in Childhood and the Environment) [28, 66-68] as well as the German Bitterfeld study [29, 69] revealed an increased occurrence of asthma in metropolitan polluted areas compared to less urbanized areas.

Some methodological considerations must be taken into account when interpreting data based on international comparisons regarding the prevalence of allergic diseases. First, relatively large numbers of participants from each selected country/region are required as well as high participation rates. Second, standardized data collection procedures are needed, which may be difficult to achieve because of cultural and linguistic differences. The wording of items on wheezing and asthma and the content of the diagnostic labels may differ considerably between surveys performed across Europe [25]. Differences in diagnostic procedures probably contribute to the West-East gradient in prevalence of allergic diseases [25]. Different awareness of allergic diseases in the different countries both from the patients and health care workers may also be of importance.

2.2.2 West-East gradient in allergy prevalence

Numerous population-based studies have shown an increase in allergy prevalence in developing countries similar to those earlier noted in developed areas [23, 70-72]. Although the prevalence of these illnesses in developing countries is still considered lower than in industrialized ones, allergic disorders are becoming an important public health problem. The rapid increase in prevalence of hay fever and atopic sensitization among schoolchildren from former Eastern Germany [31, 69], which was noted after the reunification, points towards an important role of environmental and lifestyle exposures, rather than genetic factors, for development of allergy. Substantial changes in lifestyle have occurred in many former socialistic countries, including Russia, after their transformation into market economies [3, 8, 23, 73-78]. However, only ISAAC [1, 23] as well as a small number of international [2, 11, 33, 35, 37-39, 71, 79] and domestic studies [32, 40, 80] provide data on allergy prevalence in these areas.

Only a few epidemiological surveys, focusing on allergy in Russian children have been performed [11, 32, 34-38, 40, 81]. Some of them are questionnaire-based prevalence studies of asthma and allergic diseases, which do not always include clinical examinations of the children or laboratory measurements. An epidemiological study recently performed

in Saint-Petersburg focusing on bronchial asthma and allergic rhinitis among adult population has clearly indicated that the prevalence of allergy is higher than official statistics [42]. An underdiagnosis of allergic outcomes in Russia and differences in diagnostic criteria make it difficult to compare the results from the different studies, although such data could be useful for indicating important causal or preventive factors.

Two recent Russian-Norwegian epidemiological studies on allergy in children [36] and in adults [41] put in question the common belief on lower prevalence in Eastern countries. It was shown in both studies that asthma symptoms caused by external factors, as well as respiratory symptoms, were higher among Russian children [36] and that IgE-sensitization was more common among adults in Russia than in Norway [41]. This discrepancy might reflect a different awareness of allergies in the two countries and points to a need for objective markers of atopy when comparing prevalence in different populations [41].

The ISAAC study as well as some international studies focusing on the Russian population [23, 32-36, 40, 80] showed that the prevalence of allergic conditions in Russian children only differed slightly from the one observed for Western countries.

2.3 RISK FACTORS

2.3.1 Genetic factors

It is well-established that interactions between numerous environmental influences and the genetic predisposition make asthma and allergy complex diseases, in which the effects of single genetic or environmental factors may be hard to detect [82]. Thus, the influence by different environmental factors depends on the individual's genetic background and visa versa [83-86]. It is generally agreed that genetic predisposition is of importance for the development of asthma, and probably a number of alterations in different genes contribute [82]. Several studies show that not only maternal, but paternal history of asthma and allergic diseases might be strongly significant as risk factors for the development of childhood asthma and, in some cases, the genetic contribution of the father appeared stronger [87, 88]. Similar conclusions have been reached concerning the development of childhood eczema, postulating that the risk of eczema development arises according to the type of atopic diseases in their parents [89-91].

Male gender is strongly associated with an increased risk of asthma and wheezing in children [92-97]. The same gender disparity is seen with respect to atopic sensitization in children [98]. It is probably that genetic factors contribute to this increased susceptibility.

2.3.2 Environmental factors

“Hygiene hypothesis”

It has been reported that children from areas with higher standard of living and presumably more "hygienic" environments are more likely to develop atopic diseases than children raised in economically disadvantaged environments [1, 2, 35, 41, 56]. Associations between the so-called “Western lifestyle” and prevalence of atopic disorders in children have contributed to the “Hygiene hypothesis” that was first proposed by Strachan in 1989 [99]. A key element of the hypothesis postulates a decreased exposure to infectious agents in early life caused, to some extent, by improvements of the living standards [2, 31]. The “Hygiene hypothesis” originally referred to the reciprocal model of T helper (Th) Th1/Th2 regulation, which has recently been disputed. Not all findings support the “Hygiene hypothesis”. Infections of the respiratory tract have also been identified as risk factors for wheeze and asthma. This discrepancy may in part be attributable to the phenotype of wheeze under study, early transient wheeze being positively associated with infections, whereas the atopic phenotype may be protected by increased infectious exposure. Furthermore, the type of infection may play a role [100].

It has been postulated that apart from the history of infections in early life, the wide spectrum of indoor and outdoor allergen exposures might play an important role in the etiology of allergic disorders, causing considerable variations in the prevalence between countries [8-10, 75, 77, 78, 101-103]. In light of these considerations, the possible impact of improved living standards for development of allergy remains the subject of international scientific interest, particularly for researchers studying allergy morbidity in developing countries [3].

Environmental tobacco smoke (ETS) and maternal smoking during pregnancy

Studies on the effects of parental smoking on childhood asthma show that involuntary smoking, particularly maternal smoking, is an independent risk factor for childhood allergic diseases, especially occurring in first years of life [95, 104-110]. It is difficult to distinguish the independent contributions of prenatal and postnatal maternal smoking. However, even in absence of subsequent postnatal ETS exposure, *in utero* exposure to maternal smoking significantly increased the risk of doctor-diagnosed asthma, asthma symptoms, and asthma severity later in a child’s life, as well as most of the wheezing outcomes [111]. ETS may also act as an adjuvant factor for bronchial hyperreactivity [112].

Housing conditions

The role of indoor moulds or dampness for respiratory functioning has recently been highlighted [68]. It has been shown that signs of dampness in the home are associated with respiratory symptoms and asthma [113]. Moreover, findings from a prospective study of adolescents suggest that a humidifier in the home may contribute to the onset of asthma [114]. Although it is difficult to eliminate publication bias from studies in this area, recent literature reviews support these findings [68, 86, 115, 116]. Furthermore, factors related to

renovation activities in the living area of the child, such as painting, installation of certain interior materials, etc., have been related to development of allergic diseases, particularly respiratory allergy [32, 117]. This may indicate a negative influence by certain chemical emissions on development of childhood asthma and allergic diseases, but the role of specific compounds has not been elucidated [117].

Outdoor environmental pollution

An adverse effect of traffic-related air pollution on respiratory health of children, particularly with respect to changes in lung function, has been recently emphasized [118-120]. However, the results of studies on urban air pollution and allergic diseases remains inconclusive [121-130]. Traffic-related air pollution has been reported to be positively associated with sensitization to pollen and other outdoor allergens as well as with elevated levels of total IgE in children [124-126]. Some studies revealed associations between exposure to traffic-related air pollution and exacerbation of asthma and asthmatic symptoms [121-123]. Experimental studies on air pollution and allergic diseases are mostly focused on the ability of pollutants to alter immunoglobulin production [131].

2.3.3 Lifestyle factors

Place of birth

The prevalence of asthma appears to be increased in children who migrated from countries with low asthma prevalence to places of high prevalence [132-134]. The migration process leads to substantial changes in environmental and lifestyle exposures, including dietary alterations, which could possibly be responsible for this effect [132, 134, 135].

Breastfeeding

The effect of prolonged breastfeeding, particularly exclusive, on development of allergic diseases in children remains controversial. Recently published results from BAMSE (B = Children; (Barn), A = Allergy, M = Environment; (Miljö), S = Stockholm, E = Epidemiological survey) confirm the protective role of breastfeeding for at least four months on development of asthma and eczema in children [91, 136]. However, opposite results with respect to asthma and eczema have been reported in some studies focused on children with allergic heredity [137, 138]. A protective role of breast milk for development of asthma and eczema might be explained by a reduced risk of airway obstruction induced by infections as well as by a promotion of gut colonization by intestinal microbes thought to induce a shift toward a Th1-predominant cytokine response, which possibly promotes development of oral tolerance [139-147].

Age of day-care attendance, family size, crowdedness

Early admission to day-care center may prevent development of asthma in late childhood due to an increase in the rate of cross-infection between children. Nowadays cross-infection is limited by general decrease in family sizes and higher standards of personal hygiene.

However, the data on associations between day-care attendance and risk of wheezing remain conflicting [148-155]. It has been hypothesized that cross-infection from older siblings may reduce the risk of allergic diseases. Associations between atopy and family structure have been found in many studies, although the mechanisms of the relationship are not fully understood [156]. It has also been proposed that the sibling effect originates *in utero*, rather than postnatally [157].

Use of antibiotics

It has been suggested that the treatment of infant infections, e.g. by using a broad spectrum of antibiotics, leads to alterations of the intestinal flora impairing Th1 immune responses, and promoting a Th2 polarized immune deviation that enhance the development of allergic diseases and asthma. However, the epidemiologic evidence on this association is incoherent [158-163]. In a recent meta-analysis [161], only pooling the results from retrospective studies suggested a positive association between antibiotic exposure in early life and subsequent development of asthma. On the other hand, the results of prospective studies did not confirm this association, which might indicate that the methodological quality of the studies and differences in analytical approaches influenced the results.

2.3.4 Farming-related factors

It has been shown that children brought up in a rural area, particularly those residing on farm and engaged in farm work, have a lower risk of hay fever and atopic sensitization [64, 164-166]. For asthma and wheezing the results appear inconsistent [65, 167], and recent findings regarding eczema mostly failed to show a protective effect by farm exposures [8, 168]. A number of studies have found protective effects by agricultural exposures, particularly work in stables, contacts to livestock and poultry, [164, 165, 168], consumption of farm milk, especially unboiled, and self-grown food [8, 63, 65]. The timing of exposure may be of great importance since the effect of farm environment on development of allergic diseases seems to vary by age and the protective effect of exposure before 1 year of age may reverse if exposure occurred later in life [26, 164, 169, 170].

The effect of air pollutants from wood combustion on respiratory pathology, including asthma and allergy, has been highlighted in several studies, but results appear inconclusive [171-174]. Some studies, mostly focused on allergy among rural populations, showed that children in families using wood for heating and cooking had significantly lower prevalence of hay fever, atopy, and bronchial hyperresponsiveness than children living in homes with other heating systems [175]. However, it is possible that use of wood for heating was a proxy for certain types of farming also involving exposures to protective factors for allergy in children [176]. An accumulating body of epidemiological evidence indicates that children are more susceptible to wood smoke than adults and exposure occurring early in life may result in decreased pulmonary function, increased severity and frequency of wheezing, as well as in increased incidence, severity and duration of acute respiratory

infections [171-173]. Unprocessed solid fuels are still widely used in nearly half of the world's households for cooking and heating, mostly in developing countries, leading to substantial indoor air pollution in poorly ventilated houses, particularly affecting children's health [171, 172].

2.3.5 Infections

In attempts to identify risk factors behind the geographical differences in allergy, exposure to a variety of microorganisms, particularly occurring early in life, was considered to be of importance. It has been suggested that a wide spectrum of microbes may be involved in the development of allergic diseases by promoting the maturation of the immune system and diverting the immune response in either the Th1 or Th2 direction [11, 177-181]. Certain viral infections during infancy have been implicated as potentially responsible for the development of the asthmatic phenotype [182, 183]. For example, respiratory syncytial virus (RSV) has been associated with asthma in children [184]. However, other viruses, such as herpesviruses, may also be of importance, but their possible effect has been studied only to limited extent [18, 20-22, 185, 186]. These viruses are ubiquitous pathogens, but primary infection is often delayed in developed countries. They are frequently reactivated and may affect the immune system by increasing the Th1 or Th2 immune response [187-189].

EBV

Previous epidemiological and serological surveys have not shown consistent results regarding the role of EBV infection in the development of allergy [22, 185, 186]. Rystedt and colleagues [185] found that adult patients with atopic dermatitis had elevated titers of antibodies against EBV virus capsid antigen (VCA), compared with controls. Likewise, increased titres of EBV antibodies have been reported in children 5 to 18-years old with asthma, atopic eczema or rhinoconjunctivitis, compared with non-atopics [22]. On the other hand, epidemiological studies focusing on allergic diseases in infancy and young preschool age, revealed significant negative associations between seropositivity to EBV and IgE-sensitization, particularly in children up to 6 years of age, whereas in older children a reverse relation was found [18, 22, 190].

EBV titers can reflect basic immunoregulatory disturbances. Human B cells, once transformed by EBV, produce and use interleukin-5 (IL-5) in an autocrine fashion to induce chronic eosinophilic inflammation and also produce IL-4, which has an important role in promoting the production of the IgE-antibodies. Thus, EBV infection may theoretically lead to production of some major ILs described in allergy [191-193]. However, in the allergic child B-cells harboring EBV and expressing EBV antigens could be activated, increasing the antigen load and antibody response. It is uncertain whether the elevated EBV titers are a sign that EBV causes the allergy or the result of the allergy.

Recent findings from a study on murine gammaherpesvirus 68 or murine cytomegalovirus, which are genetically similar to the human pathogens Epstein–Barr virus and human Cytomegalovirus [194] suggest that herpesvirus latency may sculpt the immune response to self and environmental antigens through establishment of a polarized cytokine environment. In other words, herpesvirus infection triggers systemic, profound immune modulation, with the potential to significantly alter the kinetics and nature of host response to foreign antigens. Latency-induced protection is not antigen specific but involves prolonged production of the antiviral cytokine interferon- γ (IFN- γ) and systemic activation of macrophages. This effect may extend beyond protection from pathogens, but also explain the reduction of IgE-sensitization to environmental allergens, suggesting that prolonged secretion of Th1-type cytokines during latency may inhibit the development of Th2-driven immune pathology [194]. In addition, human EBV is constantly shed after the primary infection, which may add to the immune modulation.

CMV

Despite great interest in the effect of microbial exposure on the development of allergic diseases, there are only few studies addressing the relation between CMV infection and subsequent development of allergic disorders in children, and the results are inconclusive [19-21, 195-197]. Some of the studies postulate a protective effect of CMV on allergy development referring to the “Hygiene hypothesis”, particularly to the balance in Th1/Th2 immune response, and suggest that the multiple and chronic ligation of Toll-like receptors with microbial components may explain the inverse associations between the chronic viral infections and allergy [197, 198]. Other studies question the idea of a protective effect by viral exposure indicating that reactivation of CMV may contribute to the development of hypersensitivity [195, 196], atopic dermatitis or sensitization to common allergens [21].

A greater degree of expansion of CD45RO memory T cell production has been shown in atopic than in nonatopic children [17]. It has also been described that CD45RO is usually expressed in response to CMV antigens [199]. The memory cells produce 10-fold more IFN- γ compared to naive T cells [200] and may divert Th1/Th2 balance to the Th1 direction. An early CMV infection could thus be of importance for the development of atopy [17]. However, in recent studies neither a difference in the Th1 response nor in seroprevalence to CMV between atopic and nonatopic children was noted [20, 21]. These studies, however, do not consider viral interactions that may play important roles in skewing the immune response and affecting the subsequent development of allergic diseases. Another possible explanation of the inconsistency in results seen in different populations may relate to different age of infection resulting in variable modulation of the immune system [197].

A complicated interplay between infections, favoring contradictory immune response, such as CMV and EBV, and the immune system, was suggested to be one of the explanations of various controversial results. These two viruses may theoretically be counteracting the

effect of each other on the immune system by their different Th1 and Th2 activations [201]. Recent studies have shown that the human immune response to CMV includes positive staining of T cells for IL-2, tumor-necrosis factor (TNF)- α , IFN- γ , and small amounts of IL-4 [202], suggesting that CMV elicits a prototype Th1 response. EBV infection, on the other hand, may transform human B cells, resulting in production of IL-5, which in turn may induce a chronic eosinophilic inflammation, promoting allergy [191]. Since Th1-type cytokines inhibit the production of Th2-type cytokines and vice versa [203], the immunological aspects of co-infection with the two viruses might be of specific interest. This may also contribute to explaining the heterogeneous findings in different studies on the effect of breastfeeding and underlines the importance of studies of interaction between viral infections.

3 AIMS

The overall objective of this work was to elucidate the role of various environmental and lifestyle factors, including chronic viral infections, for development of allergic diseases in children of preschool age from Stockholm, Sweden and Saint-Petersburg, Russia. The specific aims were:

Part 1

- To assess whether Epstein-Barr virus seropositivity is related to allergic diseases and sensitization in Swedish children at the age of four years [I].
- To clarify the role of chronic Cytomegalovirus infection in development of allergic conditions and sensitization in children [II].
- To examine whether a potential interaction between Epstein-Barr virus and Cytomegalovirus infections influences the development of allergic diseases in children [III].

Part 2

- To provide internationally comparable data on the prevalence of asthma, allergic rhinitis, eczema and sensitization among Russian children of pre-school age [III].
- To study the influence of certain environmental exposures, such as indoor and outdoor air pollution, on development of allergic diseases in Russian children [III].
- To assess the role of exposure to farm-related environmental factors for development of allergic diseases among urban Russian children [IV].

4 METHODS

Part 1

4.1 COHORT STUDY [I, II].

4.1.1 Study population

The Swedish study BAMSE is based on an ongoing follow-up of children from Stockholm, aimed at elucidating the relation between exposure to various environmental or lifestyle factors during early childhood and development of atopy. BAMSE was designed as a longitudinal prospective birth cohort using parental questionnaires to assess the health of the child as well as various environmental and lifestyle exposures accompanied by a clinical examination, including collection of blood samples. All children born in predefined areas of Stockholm from February 1994 to November 1996 were invited to participate in the study [95]. Among 7,221 children born during this period, 3,132 children were excluded from the cohort due to different reasons leaving 4,089 new-born infants (2,065 boys and 2,024 girls) in the final BAMSE birth cohort. The non-participation was mostly due to wrong address information (n=477) and individual unwillingness of the family to participate (n=1,399). Additionally, children were actively excluded from the cohort due to insufficient parental knowledge of Swedish (n=331), plans to move within one year (n=699), serious illness of the child (n=57) or if an older sibling had already been included in the study (n=169). Neither non-participants nor actively excluded children differed substantially from the study group in relation to the main exposures [95].

The parents of the included children answered questionnaires concerning various environmental factors, as well as symptoms and health outcomes during different periods of the life of their child. By the year 2002 when the EBV / CMV studies began, four questionnaires had been answered by the parents, i.e. when the children were about two months (Q₀), one year (Q₁), two (Q₂) and four (Q₄) years old.

All 3,742 children whose parents answered Q₄ were invited to a clinical examination and collection of blood samples. For different reasons, 1,128 children were not sampled. Among these, 86 had moved out of Stockholm and 291 families did not answer the invitation for unknown reasons. It was not possible to draw blood from 350 children who took part in the clinical examination, because only one “try” was allowed, and 401 families refused blood sampling. Thus, the BAMSE cohort at age four comprised 2,614 children with Q₄ data available from whom blood samples were collected. The participation rates as well as the median age of the children at the different stages of the BAMSE project are given in Table 1.

Table 1. Number of children followed in the prospective BAMSE birth cohort and participation rate at different stages of data collection.

Data / material available	Median age	Number of participants (%)
Questionnaire-0	2 months	4089 (100)
Questionnaire-1	1 year	3925 (95.9)
Questionnaire-2	2 years	3843 (93.9)
Questionnaire-4	4 years	3742 (91.5)
Blood samples	4,3 years	2614 (63.9)

Among the four-year-old children from the BAMSE birth cohort with serum samples available, samples from 33 children were excluded due to insufficient volume of serum, leaving totally 2,581 samples for serology analyses of EBV and CMV IgG-antibodies. For the EBV serology analyses, another 20 samples were excluded since the results of the assay were indeterminate despite repeated examination. Thus, 2,561 of the children with Q₄ and serology data available were included in the final study group for assessment of EBV seroprevalence (paper I) and 2,581 for assessment of CMV seroprevalence (paper II) in relation to development of allergic conditions.

4.1.2 Allergen-specific IgE analyses

The serum samples collected within the BAMSE study were analysed at the Department of Clinical Immunology at the Karolinska University Hospital, Stockholm, Sweden. Allergen-specific IgE-antibodies were measured using Phadiatop[®] (a mix of common inhalant allergens: cat, dog, *Dermatophagoides pteronyssinus*, horse, birch, mugwort, timothy, *Cladosporium herbarum*), and fx5[®] (a mix of common food allergens: cow's milk, hen's egg, wheat flour, peanut, soy bean and codfish), with ImmunoCAP, Phadia AB, Uppsala, Sweden. An IgE-antibody value of ≥ 0.35 kU_A/L was regarded as positive.

4.1.3 Serological methods

EBV serology [I]

IgG-antibodies to the EBV VCA remain lifelong after the primary infection. To investigate the EBV seroprevalence these antibodies were detected using an indirect immunofluorescence assay (IIF) [204]. Repeated analyses to confirm the validity of the

serological results were performed for selected samples. All samples examined in 6 of 20 IIF assay sets showing unusually high or low titers of seropositivity were selected, as well as 5 positive and 5 negative samples from each remaining examination set. The validity of the IIF assay was thus assessed for a total of 709 samples and showed an agreement of 0.94 using the kappa-statistic.

CMV serology [III]

The commercial enzyme-linked immunosorbent assay Enzygnost® Anti CMV/IgG (Dade Behring, Marburg GmbH, Germany) was used for determination of CMV IgG-antibodies. The analysis was performed according to the instructions from the manufacturer. The Anti-CMV Reference positive and negative samples provided by the manufacturer were used as reference samples. All reference and test samples were prediluted 1:20 with sample buffer. The quantitative evaluation was performed by the α -method and an optical density of 0.25 was chosen as a cut-off for positive results, both according to the manufacturer. Repeated analyses to confirm the validity of the Enzygnost® Anti CMV/IgG results were performed for examinations showing unusually similar results for all sera included in one run. There was no interassay variation concerning serostatus.

4.1.4 Health outcomes

In the BAMSE study, at 4 years of age, health outcomes were defined as follows:

- 1) **asthma** - at least 4 episodes of wheezing during the preceding 12 months or at least 1 episode during the same period together with treatment of inhaled corticosteroids without an ongoing cold [205].
- 2) **suspected allergic rhinitis** - runny, itchy or blocked nose during the preceding 12 months without an ongoing cold [48].
- 3) **atopic dermatitis** - an itchy rash with typical distribution ongoing for at least two weeks and dry skin; and/or doctor's diagnosis of eczema [90].
- 4) **respiratory diseases** (pneumonia, bronchitis and RSV infection) - all diagnosed by a doctor. The method used by the doctor to establish the diagnoses was not specifically requested.
- 5) **sensitization** was defined as a presence of IgE-antibodies against a mixture of common inhalant or food allergens at a level of ≥ 0.35 kU_A/L.

4.1.5 Statistical methods

Odds ratio (OR) estimates, and corresponding 95% confidence intervals (CI) for asthma, atopic dermatitis, suspected allergic rhinitis, sensitization and respiratory tract infections were obtained using logistic regression with adjustment for potential risk factors for allergic diseases. These included parental allergy, maternal smoking, maternal age at birth of the child, breastfeeding, and parental educational level. Multinomial logistic regression was used for assessment of outcomes with multiple categories. A likelihood ratio test was

performed for estimation of the interaction between CMV and EBV with respect to specific IgE antibodies to common airborne and food allergens. The statistical package STATA 7.0 was used for analyses (STATA Statistical Software. Release 7.0. College Station; Texas, USA: STATA Corporation).

As a reference group for assessment of seroprevalence to EBV the children negative to EBV IgG-antibodies were chosen (paper I). When computing the odds ratios, the effect of seropositivity to CMV (paper II) was assessed using all children who were seronegative to CMV as a comparison group, whereas for the effect of dual seropositivity to CMV and EBV (paper II) as well as single seropositivity to CMV or to EBV, children who were seronegative to both CMV and EBV were used as a reference group (Fig. 2).

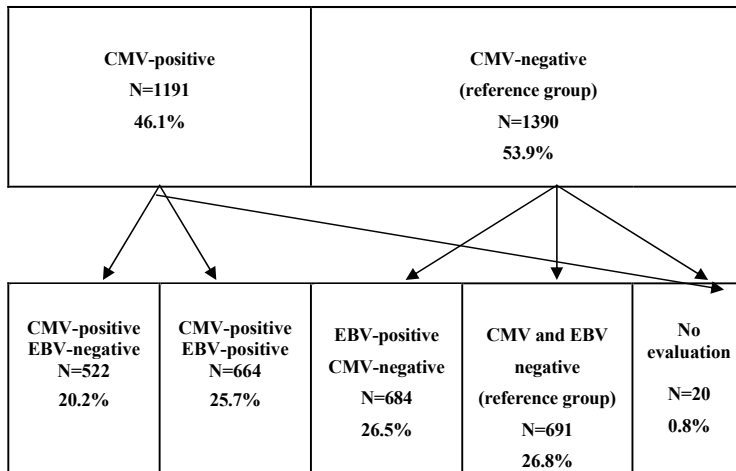


Figure 2. Number of children analyzed for CMV and EBV serostatus in the prospective BAMSE birth cohort.

Part 2

4.2 CROSS-SECTIONAL STUDY [III, IV]

4.2.1 Study population

The study population for the survey RADUGA (an abbreviation of the Russian expression for “Prevalence of Allergy in Pre-school Centers in the City of Saint-Petersburg”) comprised children aged 2-7 years, attending day care centers in three different parts of Saint-Petersburg. Two urban and one suburban district included areas with different traffic exposures, living conditions, and socioeconomic status of the families. In total, 32 day care

centers were selected. Two districts with high levels of traffic-related air pollution, i.e. “urban districts” (Admiraltejsky and Centralny) included 12 and 15 day care centers, respectively, primarily next to streets with heavy traffic. The “suburban district” with lower levels of air pollution (Primorsky) included 5 day care centers located further away (50-100 m) from streets with heavy traffic and surrounded by apartment buildings.

In the beginning of September 2003 families of all 2–7 year old children attending the selected day care centers were invited to participate. A description of the selection and participation of the study subjects is presented in Figure 3. Totally, 1,702 families out of 1,999 (85.1%) completed the questionnaires. Most of the refusals to participate were based on unwillingness of the parents. However, in some day care centers the low level of participation appeared to be due to a lack of interest of the administration and teaching staff. The mean age at survey participation was 4.5 (standard deviation 1.2) years with a sex male/female ratio of 0.94. Internal nonresponse/missing rates with regard to specific items in the questionnaire were $\leq 3\%$.

All 1,702 children with questionnaire data were invited to participate in a clinical examination that was performed one year later (October 2004 – June 2005). The invitation was accepted by 829 families (48.7%) and 566 children (33.3%) provided blood samples. Among 1,136 children not sampled, 423 had moved out of Saint-Petersburg, 527 actively rejected examination and 132 families did not respond to the invitation for unknown reasons. It was not possible to draw blood from 10 children who took part in the clinical examination. Another 44 families, originally agreeing to put the children through the doctoral check-up, refused the sampling.

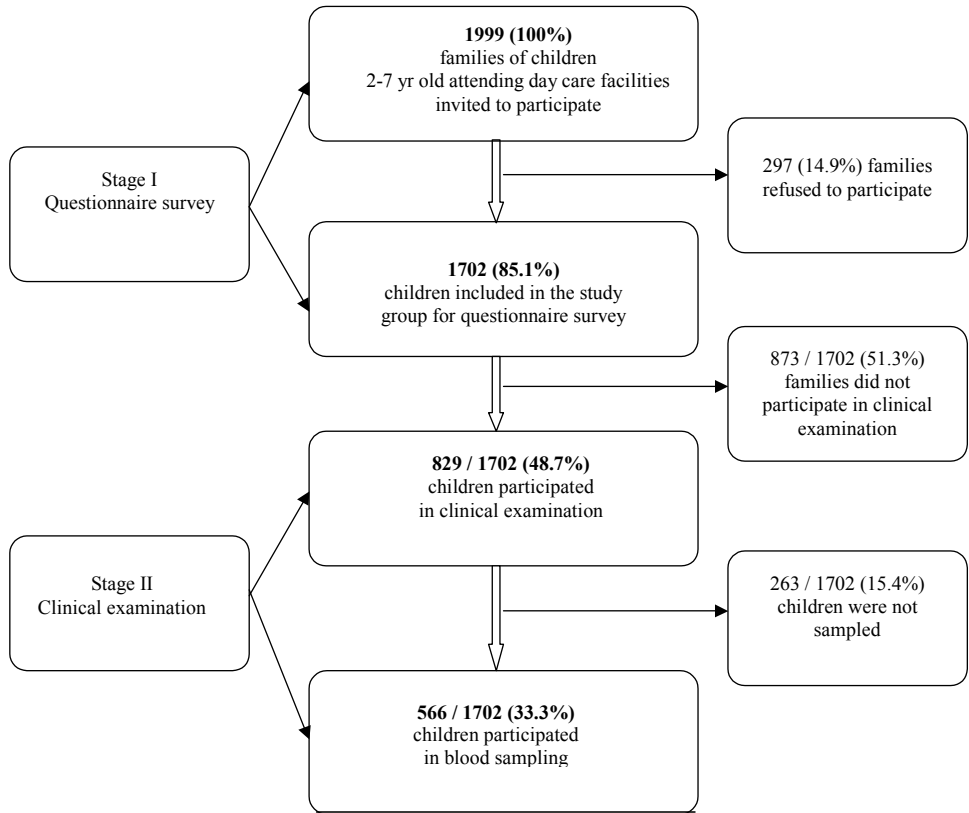


Figure 3. Number of children included in the cross-sectional study RADUGA and participation rates at different stages of data collection.

4.2.2 Questionnaire survey

RADUGA is a cross-sectional study using a detailed parental questionnaire based mostly on the validated PARSIFAL [9] and BAMSE [206] studies. The RADUGA questionnaire dealt with assessment of “current” (during the preceding 12 months) and previous (ever after birth of the child) exposure to various indoor and outdoor environmental factors, lifestyle characteristics, diet, child’s history of allergic manifestations, and family history of allergic diseases.

To avoid possible misinterpretations of the medical terms subsidiary information containing the Russian labeling for allergic diagnoses was included in the questionnaire. Therefore, the international term “rhinoconjunctivitis” was additionally denoted in the questionnaire as “hay fever” and “pollinosis”. “Atopic eczema” was also named

“neurodermitis”, “atopic dermatitis” and “endogenous eczema”. The diagnosis “obstructive bronchitis” was interpreted as “asthmatic bronchitis” as well.

The questionnaire was carefully piloted in one day care center, which was not further included in the main study, and then revised according to the comments received. For the main study the questionnaires were distributed and collected by the administration and teaching staff of the day care centers during the time period from September to December 2003.

4.2.3 Clinical examination and blood testing

The clinical examination was performed by doctors (paediatrician-allergologists) and specially trained nurses recruited from the largest children’s hospital in Saint-Petersburg and included a doctor’s check-up of the child accompanied by interview with the parents, measurement of weight and height, peakflowmetry and blood sampling. During the interview additional data on the health status of the child and family members were collected, mainly focused on occurrence of allergic diseases, symptoms and use for allergy-medicine. Informed consent was obtained from the parents of each child before the examination and blood sampling. During the interview parents were asked to specify any information that was missing or unclear in their particular questionnaire.

4.2.4 Allergen-specific IgE analyses

Frozen serum samples were transported to Sweden and analysed at the Department of Clinical Immunology at the Karolinska University Hospital, Stockholm, Sweden. Allergen-specific IgE-antibodies were measured using Phadiatop® (a mix of common inhalant allergens: cat, dog, *Dermatophagoides pteronyssinus*, horse, birch, mugwort, timothy, *Cladosporium herbarum*), and fx5® (a mix of common food allergens: cow’s milk, hen’s egg, wheat flour, peanut, soy bean and codfish), with ImmunoCAP, Phadia AB, Uppsala, Sweden. An IgE value of ≥ 0.35 kU_A/L was regarded as positive.

For children defined as sensitized either to airborne or food allergens in the multi-allergen test, further analyses were made to measure IgE-antibodies against the single allergens listed above. In addition, IgE-antibodies to cockroach (*Blatella germanica*) were determined for all children who provided blood samples. Totally, 563 sera out of 566 were available for IgE-determination since samples from 3 children could not be analysed due to insufficient volume.

4.2.5 Health outcomes

Children were defined as having current (during the preceding 12 months) **symptoms** of:

- 1) **wheezing** - report of at least 1 episode of wheezing [9];
- 2) **rhinitis** – defined as sneezing, stuffy or runny nose accompanied by itchy eyes occurring without the child having a cold at the same time [206];
- 3) **eczema** - the child had had an itchy rash with typical distribution (face/outer limbs/folds of elbows or behind the knees/wrists or fronts of ankles [9].

Children reported to ever had been diagnosed with asthma, or, more than once, with obstructive bronchitis were considered to have **physician-diagnosed asthma**. The classification of **physician-diagnosed allergic rhinitis** and **eczema** was also based on parental reporting in the questionnaire.

4.2.6 Statistical analyses

Statistical analyses were performed using the Stata statistical package (Version 8.0, Stata Corp LP, College Station, TX, USA). OR estimates, and corresponding 95% CI for asthma, allergic rhinitis, eczema and sensitization, were obtained using logistic regression and adjusted for potential risk factors. The adjustment included sex, child's age, district where the day-care center was located, mother's/father's reported asthma and/or rhinoconjunctivitis, maternal smoking during pregnancy, environmental tobacco smoking ever, maternal age at the birth of the child, breastfeeding, parental educational level and number of older siblings. A likelihood ratio test was performed to estimate the interaction between various exposures with respect to the outcomes of interest.

5 RESULTS

5.1 PAPER I: EBV SEROPOSITIVITY AND ALLERGIC DISEASES

To assess whether the children with EBV (paper I) and / or CMV (paper II) serology available differ from those who did not participate in blood sampling the distribution of socio-economic characteristics in participants / non-participants was assessed (Table 2). There was a higher prevalence of bronchitis among the participants than among the non-participants, and a similar tendency was observed for asthma and suspected allergic rhinitis. A higher prevalence of breastfeeding among participants was also seen compared to non-participants.

Table 2. Socio-economic characteristics and occurrence of allergic outcomes and respiratory pathology in 1,181 “non-participants” from the prospective BAMSE birth cohort in comparison to 2,561 children in the study population for the EBV/CMV studies.

Risk factors and outcomes	Non-participants N=1,181	Study population N=2,561	OR (CI 95%)
Gender			
Female	580 (49.9)	1273 (49.3)	1.0
Male	581 (50.0)	1308 (50.7)	1.01 (0.89-1.21)
Maternal age			
>25 years	1025 (88.3)	2252 (87.3)	1.0
≤25 years	131 (11.3)	320 (12.4)	1.11 (0.90-1.42)
Unknown ¹	5 (0.4)	9 (0.4)	
Breastfeeding			
No breastfeeding	42 (3.6)	57 (2.2)	1.0
<4 months	70 (6.0)	159 (6.2)	1.61 (0.92-2.71)
≥4 months	1017 (87.6)	2325 (90.1)	1.72 (1.01-2.52)
Unknown ¹	32 (2.8)	40 (1.6)	
Maternal smoking			
Never	904 (77.9)	2097 (81.3)	1.0
During pregnancy only	25 (2.2)	55 (2.1)	0.90 (0.61-1.51)
Ever after birth of child	189 (16.3)	377 (14.6)	0.92 (0.71-1.03)
Unknown ¹	43 (3.7)	52 (2.0)	
Educational level of the parents²			
High	626 (53.9)	1357 (52.6)	1.0
Middle	300 (25.8)	701 (27.2)	1.11 (0.91-1.31)
Low	229 (19.7)	511 (19.8)	1.01 (0.92-1.20)
Unknown ¹	6 (0.6)	12 (0.4)	

Table 2. (continued)

<u>Risk factors and outcomes</u>	Non-participants N=1,181	Study population N=2,561	OR (CI 95%)
<u>Allergic diseases:</u>			
Asthma			
No	1088 (93.7)	2369 (91.8)	1.0
Yes	67 (5.8)	193 (7.5)	1.28 (0.96-1.69)
Unknown ¹	6 (0.5)	19 (0.7)	
Suspected allergic rhinitis			
No	1039 (89.5)	2246 (87.0)	1.0
Yes	115 (9.9)	298 (11.6)	1.19 (0.95-1.50)
Unknown ¹	7 (0.6)	37 (1.4)	
<u>Respiratory tract infections:</u>			
Pneumonia			
Never	1082 (93.2)	2465 (95.5)	1.0
Ever	21 (1.8)	46 (1.8)	0.91 (0.63-1.62)
Unknown ¹	58 (5.0)	70 (2.7)	
Bronchitis			
Never	1031 (88.8)	2286 (88.6)	1.0
Ever	68 (5.9)	206 (7.9)	1.35 (1.02-1.78)
Unknown ¹	62 (5.3)	189 (3.5)	
RSV infection			
Never	1082 (93.2)	2433 (94.3)	1.0
Ever	46 (4.0)	107 (4.1)	1.01 (0.73-1.54)
Unknown ¹	33 (2.8)	41 (1.6)	

¹ Subjects answered questionnaires but no answer was given to this particular item

² High parental educational level is one or both parents held degree from university; middle - one or both parents held gymnasium, technical school or college degrees; low - secondary school or lower

The total number of EBV seropositives was 1347 of 2561 (52.6%). The relation between risk factors known to contribute to development of allergic diseases and EBV seropositivity is presented in Figure 4. Among them only maternal smoking after birth of the child and young maternal age (≤ 25 yrs old) revealed significant associations. Additional adjustment for age at the time for blood sampling did not affect the results.

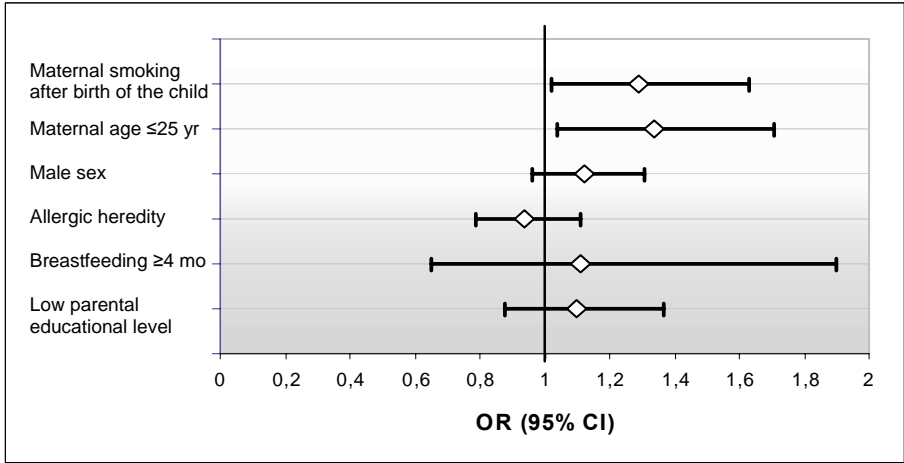


Figure 4. Odds Ratios (95% confidence interval) for seropositivity to EBV at age 4 in relation to risk factors for allergy in children from the prospective BAMSE birth cohort.

Figure 5 shows the association between EBV seropositivity and allergy, sensitization and respiratory tract infections. No clear associations were found between the seroprevalence to EBV and the allergic diseases outcomes.

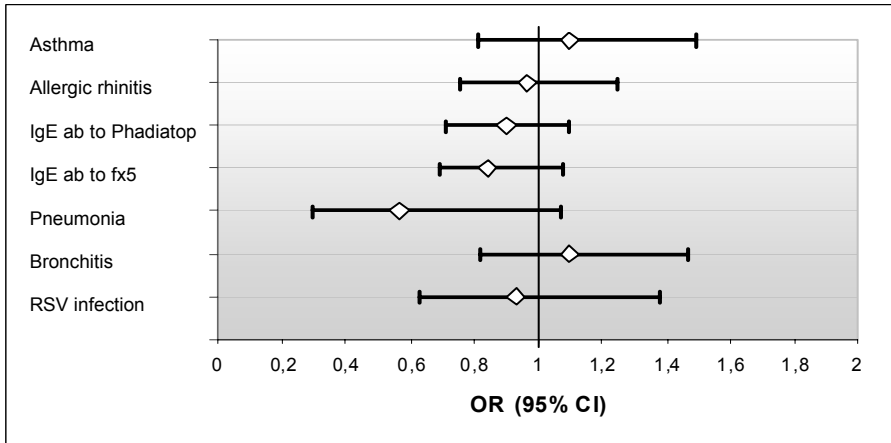


Figure 5. Odds Ratios (95% confidence interval) for outcomes of allergy, sensitization and respiratory tract infections in relation to EBV seropositivity in 4-year old children from the prospective BAMSE birth cohort.

5.2 PAPER II: SEROPOSITIVITY TO CMV, INTERACTION WITH EBV AND ALLERGIC DISEASES

The distribution of viral seropositivity is presented in Figure 6. EBV and CMV seropositivity was found in 53% and 46% of children, respectively. Among them exclusive seropositivity to these viruses was seen in 27% and 20% of the children, respectively, while 26% of them showed seropositivity to both viruses.

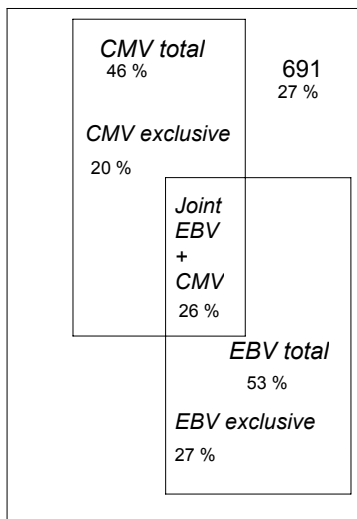


Figure 6. Seropositivity to EBV and CMV among BAMSE children at age of 4 years.

The relation between seropositivity to CMV and different risk factors for development of allergy is presented in Figure 7. The only positive associations were found for young maternal age (≤ 25 yrs of age) and long duration of breastfeeding (≥ 4 months), while large size of the living area of the child (≥ 35 m² per person) showed an inverse association.

The relations between allergic or other outcomes and total IgG to CMV are presented in Figure 8. No clear associations with the seroprevalence to CMV were apparent, but a higher prevalence of sensitization was suggested among those with CMV seropositivity.

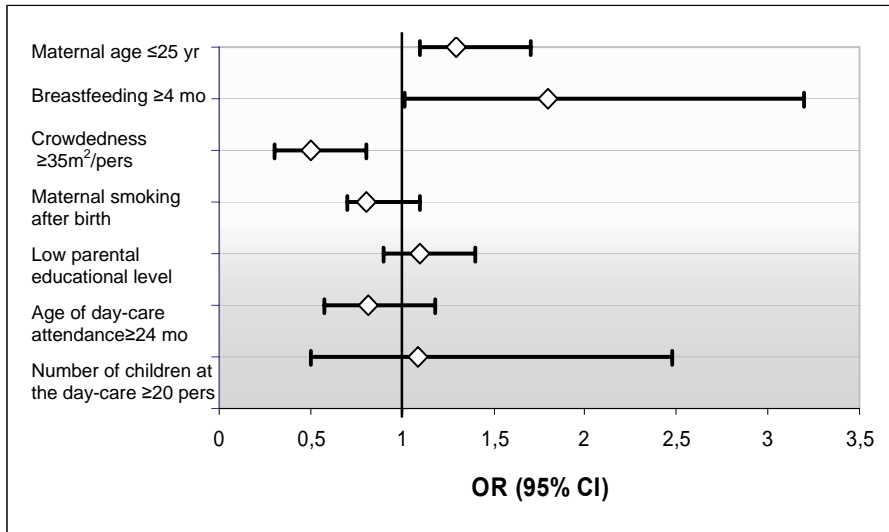


Figure 7. Odds ratios and 95% confidential interval for total seropositivity to CMV in relation to risk factors for allergy in 4-year old children from the BAMSE birth cohort.

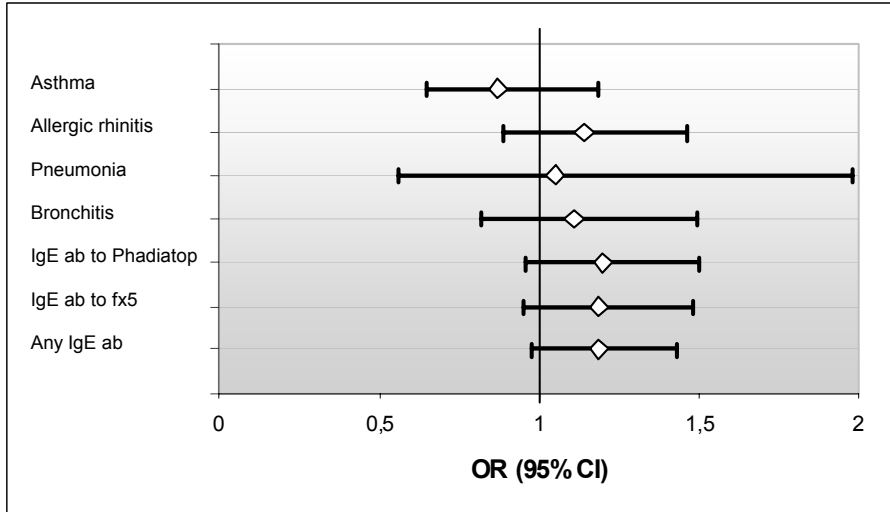


Figure 8. Odds Ratios (95% confidence interval) for outcomes of allergy, sensitization and respiratory tract infections, in relation to CMV seropositivity among 4-year old children from the prospective BAMSE birth cohort.

Single seropositivity to CMV was positively related only to young maternal age, while single positivity to EBV appeared to be associated with young maternal age, maternal smoking after birth of the child as well as with male sex. For children positive to both viruses a positive relation was found with young maternal age and a negative association – with “lack of crowdedness”.

There was no clear association between any allergic disease and single seropositivity to either CMV or EBV or to joint viral seropositivity (Table 3). However, among children seronegative for EBV those who were seropositive for CMV had an increased prevalence of specific IgE to common airborne allergens and an increased joint prevalence of IgE to airborne and food allergens. There was a borderline antagonism between CMV and EBV infection in relation to sensitization ($p = 0.05$).

Table 3. Prevalence and risk (odds ratios and 95% confidential interval) of atopic diseases as well as allergic sensitization in relation to single and joint seropositivity to CMV and EBV in children from the prospective BAMSE birth cohort at 4 years of age.

Outcomes	Children negative to both CMV and EBV N=691 (reference group)		CMV-positive EBV-negative children N=522		CMV-negative EBV-positive children N=684		CMV and EBV positive children N=664	
	Affected (%) / Not affected	Affected (%) / Not affected	Affected (%) / Not affected	Adjusted ¹ OR (CI 95%)	Affected (%) / Not affected	Adjusted ¹ OR (CI 95%)	Affected (%) / Not affected	Adjusted ¹ OR (CI 95%)
Allergic diseases:								
Asthma	50 (7.2) / 634	34 (6.5) / 486	0.8 (0.5-1.3)	0.8 (0.5-1.3)	56 (8.2) / 621	1.0 (0.7-1.5)	50 (7.5) / 611	0.9 (0.6-1.4)
Suspected allergic rhinitis	74 (10.7) / 606	64 (12.3) / 448	1.1 (0.8-1.6)	1.1 (0.8-1.6)	76 (11.1) / 599	0.9 (0.7-1.3)	82 (12.4) / 577	1.1 (0.8-1.6)
Atopic dermatitis	138 (20.0) / 553	123 (23.6) / 397	1.2 (0.9-1.6)	1.2 (0.9-1.6)	148 (21.6) / 535	1.1 (0.8-1.4)	133 (20.0) / 526	1.0 (0.8-1.3)
Specific IgE ab to common allergens:								
Phadiatop [®]	91 (13.2) / 600	96 (18.4) / 426	1.4 (1.0-1.9)	1.4 (1.0-1.9)	91 (13.9) / 589	1.0 (0.7-1.4)	99 (14.9) / 565	1.1 (0.8-1.5)
fx5 [®]	95 (13.8) / 596	95 (18.0) / 428	1.3 (0.9-1.8)	1.3 (0.9-1.8)	95 (13.6) / 591	0.9 (0.7-1.3)	99 (14.9) / 565	1.1 (0.8-1.5)
Both allergens	37 (5.4) / 654	51 (9.8) / 471	1.8 (1.2-2.9)	1.8 (1.2-2.9)	44 (6.4) / 640	1.1 (0.7-1.8)	41 (6.2) / 623	1.1 (0.7-1.8)

¹ Adjusted for maternal age, breastfeeding, gender, parental allergy, maternal smoking, and parental educational level.

To increase the specificity of the definitions of allergy phenotypes, the parental-reported symptoms were combined with IgE-sensitization to airborne or food allergens. Overall, no clear associations were observed. However, there was an association between CMV seropositivity and atopic dermatitis in combination with sensitization to food allergens among EBV-negative children ($OR_{adj} = 1.9$; 95% CI 1.2-3.2).

Part 2

5.3 PAPER III: RADUGA - ENVIRONMENTAL AND LIFESTYLE FACTORS

Participation rates

In the questionnaire survey a response rate of 85% was achieved. There was a difference in participation rates between districts/day-care centers, particularly for the suburban Primorsky district where the unwillingness to participate in the clinical examination was more pronounced. For the clinical examination, and particularly for blood sampling, the participation rates were lower, and reached 48% and 33%, respectively, among questionnaire responders.

The distribution of socio-economic characteristics and prevalence of parental-reported allergic conditions among 566 children with both questionnaire and allergen-specific IgE data as well as among those 1,136 who did not provide blood samples, but answered the questionnaire, is presented in Table 4. Some of the exposures were more prevalent among sampled children compared to non-sampled, particularly family history of allergic disorders and indoor exposure to tobacco smoke. Some outcomes, such as parental-reported current symptoms of asthma and eczema, were also more prevalent in the group with blood samples.

Table 4. Socio-economic characteristics and prevalence of allergic diseases in 566 children from Saint-Petersburg, Russia with questionnaire and immunology data available as well as in 1,136 children with questionnaire data only.

Risk factors and outcomes	Children with questionnaire data and blood samples N=566 (%)	Children with questionnaire data but without blood samples N=1136 (%)	P-value
Gender			
Female	306 (54.1)	572 (50.5)	0.15
Male	260 (45.9)	564 (49.5)	
Allergic heredity			
None	444 (78.4)	908 (79.9)	0.01
One parent	84 (14.8)	143 (12.6)	
Both parents	19 (3.4)	19 (1.7)	
District where day care centre is located			
Primorsky district	96 (16.9)	391 (34.4)	<0.01
Admiraltejsky district	190 (33.6)	259 (22.8)	
Centralny district	280 (49.5)	483 (41.5)	
Educational level of the parents			
High	325 (57.4)	704 (61.9)	0.26
Middle	212 (37.5)	372 (32.8)	
Low	25 (4.4)	54 (4.8)	
Maternal age			
>25 yr	262 (46.3)	591 (52.0)	0.08
≤25 yr	301 (53.2)	541 (47.6)	
Current environmental smoking			
No	271 (47.9)	606 (53.4)	0.04
Yes	293 (51.8)	520 (45.8)	
Use of firewood stove in the kitchen ever			
No	505 (89.2)	1007 (88.6)	0.46
Yes	60 (10.6)	122 (10.7)	
Physician-diagnosed asthma			
No	525 (92.8)	1057 (93.1)	0.24
Yes	33 (5.8)	52 (4.6)	
Current symptoms of asthma			
No	483 (85.3)	1019 (89.7)	0.03
Yes	62 (10.9)	84 (7.4)	
Physician-diagnosed allergic rhinitis			
No	532 (94.0)	1074 (94.5)	0.73
Yes	22 (3.9)	36 (3.2)	

Table 4. (continued).

Risk factors and outcomes	Children with questionnaire data and blood samples N=566 (%)	Children with questionnaire data but without blood samples N=1136 (%)	P-value
Current symptoms of allergic rhinitis			
No	518 (91.5)	1036 (91.2)	
Yes	29 (5.1)	67 (5.9)	0.72
Physician-diagnosed eczema			
No	444 (78.5)	934 (82.2)	
Yes	118 (20.9)	188 (16.6)	0.06
Current symptoms of eczema			
No	446 (78.8)	948 (83.4)	
Yes	112 (19.8)	168 (14.8)	0.03

Prevalence of allergic outcomes

Physician-diagnosed allergic diseases ever were reported for 23% of the children and 26% reported current allergic symptoms. The prevalence of physician-diagnosed asthma was 5.1%, allergic rhinitis 3.5% and eczema 18.2%, and for the corresponding current symptoms 8.9%, 5.8% and 16.7%, respectively. Only 2.7% of the children were reported to have two or more physician-diagnosed outcomes (mostly a combination of asthma and eczema), while 4.5% had two or more current symptoms (mostly asthma and eczema symptoms). More than every fourth child of the sampled subgroup was sensitized either to airborne or food allergens. Sensitization to food allergens was most prevalent (22.1%). The prevalence data appeared to be similar to the ones revealed among four-year-old children in BAMSE study (Figure 9).

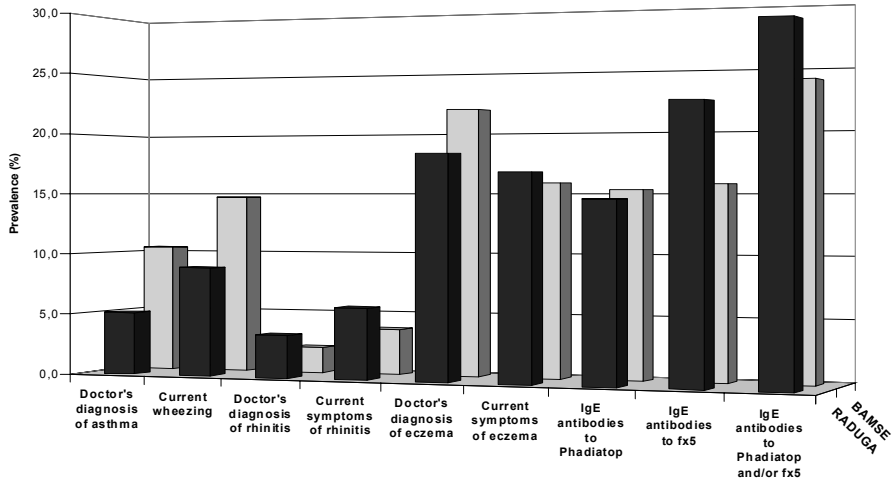


Figure 9. Prevalence of physician-diagnosed allergic diseases as well as of current symptoms among children from St Petersburg aged 2-7 years (RADUGA). For comparison, corresponding data are shown for 4-year old children from Stockholm (BAMSE).

Among children with current allergic symptoms (either wheezing, rhinitis, or eczema) 41% were sensitized to at least one of the tested allergens, while the prevalence was 25.6% in asymptomatic children.

The IgE-antibody levels against single allergens are presented in Figure 10. Half of the cockroach-sensitized children were also sensitized to house dust mites (data not shown).

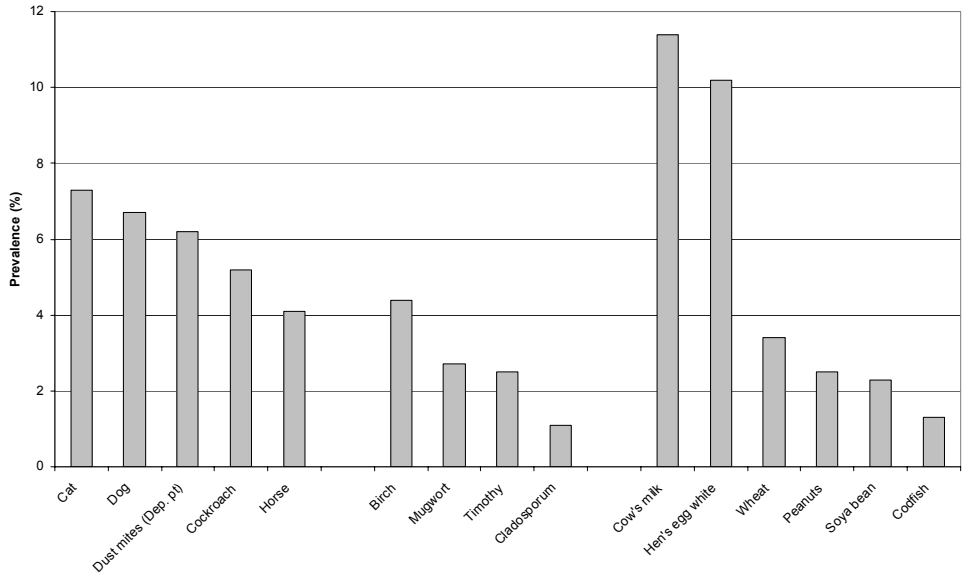


Figure 10. Prevalence of allergen-specific IgE-antibodies to single airborne and food allergens in 2-7-year old children from Saint-Petersburg, Russia.

Associations between environmental exposures and allergic diseases in RADUGA

Associations between certain environmental exposures of interest and allergy-related outcomes in the children are presented in Table 5.

Table 5. Odds Ratios and 95% confidence intervals for allergic diseases and sensitization in relation to environmental risk factors in children from Saint-Petersburg, Russia.

Risk factors	OR adjusted*						
	Physician- diagnosed asthma ever	Asthma current symptoms	Physician- diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician- diagnosed eczema ever	Eczema current symptoms	IgE antibodies to any of the allergens tested†
Current environmental smoking	1.83 (1.11-3.02)	1.51 (1.02-2.22)	1.16 (0.64-2.11)	1.85 (1.16-3.95)	0.92 (0.70-1.22)	0.99 (0.74-1.32)	1.03 (0.69-1.54)
Signs of dampness#	1.11 (0.64-1.92)	1.07 (0.70-1.64)	1.36 (0.72-2.56)	1.50 (0.91-2.46)	1.36 (0.99-1.86)	1.54 (1.12-2.11)	0.88 (0.55-1.41)
Use of gas stove at the kitchen ever	1.36 (0.62-3.00)	1.60 (0.80-3.17)	1.15 (0.44-2.97)	0.96 (0.48-1.93)	1.05 (0.69-1.61)	0.88 (0.58-1.36)	0.94 (0.46-1.93)
Use of firewood stove at the kitchen ever	2.10 (1.14-3.87)	1.46 (0.87-2.47)	2.95 (1.49-5.88)	1.82 (1.00-3.31)	1.09 (0.72-1.67)	1.35 (0.89-2.05)	1.42 (0.76-2.65)
Renovation at the child's living area	0.97 (0.59-1.59)	1.28 (0.87-1.87)	1.86 (1.04-3.34)	1.53 (0.97-2.41)	0.93 (0.70-1.24)	1.09 (0.81-1.46)	0.89 (0.58-1.36)
Age of construction After 1970	0.42 (0.14-1.29)	0.86 (0.42-1.75)	0.37 (0.10-1.40)	0.64 (0.25-1.63)	0.66 (0.38-1.14)	0.47 (0.25-0.86)	0.55 (0.24-1.22)
District where day care centre is located							
Admiraltejsky (urban)	1.08 (0.56-2.09)	2.16 (1.27-3.68)	1.06 (0.48-2.37)	0.83 (0.45-1.55)	1.09 (0.76-1.57)	0.65 (0.43-0.96)	0.85 (0.48-1.49)
Centralny (urban)	1.10 (0.61-1.97)	1.68 (1.01-2.78)	1.18 (0.59-2.38)	0.93 (0.54-1.58)	1.16 (0.84-1.60)	1.10 (0.80-1.53)	0.65 (0.38-1.11)

* Adjusted for sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding, maternal age.

† Increased level of IgE-antibodies ($\geq 0.35\text{kU}_A/L$) to either inhalant, food, or cockroach allergens.

Small or visible signs of mould in the dwelling and/or water damage inside construction.

More than 40% of the families reported current smoking in the household. It was more prevalent among people with a lower level of education and those living under less affluent conditions. Among mothers reporting current smoking, 50% also reported a history of smoking during pregnancy. Smoking among mothers was mostly associated with young age (younger than 25 years old) and to a shorter period of breastfeeding, including exclusive breastfeeding.

There were strong increases in the risk of physician-diagnosed asthma associated with exposure to ETS and use of wood stoves (Table 5). These exposures were also related to an increased risk of current asthma symptoms, physician-diagnosed allergic rhinitis and current allergic rhinitis symptoms. Furthermore, there appeared to be an interaction between ETS and wood smoke with regard to physician-diagnosed asthma. Joint exposure to ETS and wood smoke was associated with an OR_{adj} of 4.79 (95% CI, 2.25-10.2), while ETS alone had an OR_{adj} of 1.45 (95% CI, 0.84-2.52) and wood smoke an OR_{adj} of 0.87 (95% CI, 0.25-3.01).

For ETS positive exposure-response relations were observed in relation to physician-diagnosed asthma. Thus, children exposed to environmental tobacco smoke of less than 20 cig/day, showed an OR_{adj} of 1.66 (95% CI 0.99-2.76), while exposure to more than 20 cig/day was associated with an OR_{adj} of 5.66 (95% CI 2.45-13.10). Similar dose-response relationships were observed with regard to current wheezing and current allergic rhinitis symptoms.

Other indoor factors, such as signs of dampness and renovation at the child's living area showed positive associations with current eczema symptoms and diagnosed rhinitis, respectively, while living in apartments constructed after 1970 was related to a decrease in current eczema prevalence. Urban location of the day care center was associated with an increase in prevalence of current wheezing. Overall, no clear associations were seen between any of the environmental risk factors and IgE-mediated sensitization.

Associations between life-style exposures and allergic diseases

A number of lifestyle factors appeared to be associated with allergic outcomes (Table 6). Statistically significant, positive associations with various allergic manifestations were found for male sex, allergic heredity, migration to Russia after birth of the child, and use of antibiotics, particularly early in life, while a negative association was revealed for early day care admission. The strong positive association for use of antibiotics was primarily confined to those with a history of pneumonia and/or bronchitis early in life.

Table 6. Odds Ratios and 95% confidence intervals for allergic diseases and sensitization in relation to potential risk factors in children from Saint-Petersburg, Russia.

Risk factors	OR adjusted*						
	Physician-diagnosed asthma ever	Asthma current symptoms	Physician-diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician-diagnosed eczema ever	Eczema current symptoms	IgE antibodies to any of the allergens tested [†]
Male sex	2.52 (1.52-4.17)	1.52 (1.05-2.21)	0.76 (0.43-1.36)	1.31 (0.84-2.05)	1.17 (0.90-1.53)	0.85 (0.65-1.12)	1.14 (0.77-1.68)
Allergic heredity							
One parent	2.42 (1.38-4.24)	2.20 (1.40-3.46)	3.77 (2.02-7.04)	2.07 (1.22-3.52)	2.23 (1.59-3.12)	2.41 (1.72-3.39)	1.94 (1.17-3.23)
Both parents	1.79 (0.52-6.23)	3.96 (1.77-8.87)	7.95 (2.98-21.2)	2.05 (0.69-6.09)	1.89 (0.91-3.92)	2.45 (1.17-5.10)	1.72 (0.66-4.49)
Number of older siblings	1.26 (0.71-2.26)	0.98 (0.62-1.55)	1.23 (0.62-2.45)	0.59 (0.33-1.07)	1.23 (0.90-1.69)	0.93 (0.66-1.31)	0.75 (0.46-1.23)
1 older sibling	1.35 (0.49-3.71)	1.26 (0.58-2.74)	2.37 (0.87-6.45)	0.92 (0.34-2.48)	0.50 (0.23-1.08)	0.89 (0.45-1.74)	2.03 (0.76-5.36)
2 older siblings	2.41 (0.50-11.5)	2.90 (0.89-9.40)	0	1.50 (0.32-7.00)	0.69 (0.19-2.45)	0.23 (0.03-1.77)	0.37 (0.04-3.24)
3 and more							
Place of birth (outside of Russia)	15.8 (2.51-99.3)	8.46 (1.48-48.3)	0	9.21 (1.56-54.5)	2.40 (0.43-13.3)	1.12 (0.13-9.71)	0
Maternal age \leq 25 yr	1.05 (0.61-1.78)	0.91 (0.61-1.38)	0.78 (0.40-1.50)	0.68 (0.42-1.12)	0.85 (0.63-1.14)	1.01 (0.74-1.37)	0.75 (0.49-1.14)

Table 6. (continued).

Risk factors	OR adjusted*						
	Physician- diagnosed asthma ever	Asthma current symptoms	Physician- diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician- diagnosed eczema ever	Eczema current symptoms	IgE antibodies to any of the allergens tested [†]
Educational level of the parents							
Middle	0.80 (0.47-1.37)	1.04 (0.69-1.57)	0.89 (0.46-1.74)	0.63 (0.36-1.09)	0.75 (0.55-1.02)	0.98 (0.72-1.34)	1.04 (0.69-1.59)
Low	0.54 (0.12-2.36)	0.48 (0.14-1.64)	0.58 (0.07-4.51)	1.22 (0.41-3.65)	0.94 (0.47-1.88)	0.73 (0.32-1.66)	0.54 (0.16-2.00)
Exclusive breastfeeding during 4 months and more	0.97 (0.59-1.58)	0.85 (0.58-1.24)	1.38 (0.76-2.53)	1.05 (0.66-1.69)	1.02 (0.77-1.34)	0.91 (0.68-1.21)	0.85 (0.57-1.27)
Age at first use of antibiotics							
after 12 mo age	1.58 (0.86-2.89)	1.70 (1.08-2.66)	1.10 (0.52-2.32)	0.91 (0.51-1.61)	1.51 (1.09-2.08)	1.18 (0.85-1.66)	0.66 (0.41-1.06)
before 12 mo age	2.32 (1.29-4.16)	1.81 (1.14-2.89)	1.78 (0.89-3.58)	1.47 (0.85-2.52)	1.48 (1.06-2.08)	1.27 (0.90-1.79)	0.84 (0.51-1.39)
Age of admission at the day-care center							
≥2 - <3 yr old	0.51 (0.28-0.92)	0.58 (0.37-0.91)	0.60 (0.29-1.23)	1.06 (0.60-1.88)	0.95 (0.69-1.32)	0.99 (0.71-1.40)	0.93 (0.58-1.48)
≥3 yr old	0.78 (0.42-1.47)	0.83 (0.50-1.37)	0.67 (0.30-1.53)	1.32 (0.70-2.48)	0.99 (0.68-1.45)	1.16 (0.78-1.72)	0.79 (0.44-1.44)

* Adjusted for sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding, maternal age.

† Increased level of IgE-antibodies ($\geq 0.35\text{kU}_A/L$) to either inhalant, food, or cockroach allergens.

5.4 PAPER IV: RADUGA - FARM-RELATED FACTORS

About 25% of the children reported regular (once a week) contact with livestock and poultry ever after birth. Among mothers, 5% reported similar contacts during pregnancy. Farm milk was consumed by 35% of children and 10% used it regularly (once a week or more). Consumption of farm vegetables and fruits was reported for 72% of the children and most of them consumed it at least once a week.

Maternal farm-related exposure during pregnancy was positively related to the child's current symptoms of asthma and eczema as well as physician-diagnosed allergic rhinitis (Table 7). Current asthma symptoms of the child showed associations with maternal contact to pigs, rabbits and goats, while physician-diagnosed rhinitis was related to contact with horses, pigs and sheep. Current eczema symptoms were associated with contacts to livestock and, particularly with contacts to cows, pigs and sheep. Current symptoms of allergic rhinitis tended to be positively related to maternal farm activity during pregnancy, although these associations were not statistically significant. Physician-diagnosed asthma and eczema as well as sensitization showed inconsistent results.

Exposure of the child to farming animals only showed a statistically significant association for current wheezing and contact with pigs (Table 8). A similar tendency, but not significant, was seen for physician-diagnosed asthma and eczema as well as for current symptoms of asthma. Additional analyses focused on age at exposure to livestock were made but no consistent pattern of risk appeared.

Regular consumption of farm milk (Table 9) showed a significant inverse association with physician-diagnosed eczema and the trend was similar for other outcomes of allergic diseases and sensitization, except for physician-diagnosed allergic rhinitis. Consumption of farm fruits and vegetables as well as fermented food were not clearly related to any of the allergic outcomes.

Table 7. Odds Ratios and 95% confidence intervals for maternal exposure to farm animals during pregnancy in relation to allergic diseases and sensitization in children from Saint-Petersburg, Russia.

Animal contact for mother during pregnancy	OR adjusted*						
	Physician-diagnosed asthma ever	Asthma current symptoms	Physician-diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician-diagnosed atopic eczema ever	Atopic eczema current symptoms	IgE antibodies to any of the allergens tested†
Any farm animal	1.25 (0.43-3.65)	1.72 (0.82-3.58)	2.24 (0.78-6.31)	1.68 (0.68-4.15)	1.01 (0.53-1.92)	1.91 (1.08-3.37)	0.85 (0.39-1.87)
Cow	0.63 (0.08-4.83)	2.22 (0.85-5.83)	2.76 (0.73-10.5)	1.33 (0.30-5.89)	1.60 (0.68-3.74)	2.82 (1.28-6.21)	1.33 (0.43-4.09)
Hens	0.87 (0.20-3.76)	1.98 (0.88-4.49)	2.03 (0.57-7.27)	1.42 (0.48-4.18)	0.97 (0.46-2.08)	1.54 (0.77-3.09)	0.69 (0.26-1.84)
Horses	0.93 (0.12-7.43)	2.98 (0.99-8.97)	4.53 (1.13-18.2)	1.03 (0.13-8.26)	0.74 (0.20-2.71)	0.85 (0.23-3.11)	0.63 (0.12-3.35)
Pigs	1.47 (0.32-6.74)	3.39 (1.31-8.81)	4.66 (1.32-16.4)	2.63 (0.72-9.57)	0.80 (0.26-2.45)	4.13 (1.81-9.42)	1.39 (0.44-4.41)
Rabbits	2.71 (0.58-12.8)	4.21 (1.39-12.7)	1.55 (0.18-13.4)	3.21 (0.86-11.9)	0.24 (0.03-1.89)	1.53 (0.52-4.47)	0.68 (0.14-3.46)
Goats	3.62 (0.95-13.8)	5.87 (2.08-16.6)	2.98 (0.58-15.4)	3.69 (0.97-13.9)	0.25 (0.03-1.91)	1.18 (0.36-3.87)	1.29 (0.22-7.58)
Sheep	1.18 (0.14-9.59)	2.70 (0.81-8.97)	5.52 (1.32-23.1)	1.57 (0.19-12.9)	1.99 (0.67-5.95)	3.03 (1.06-8.09)	0.57 (0.06-4.40)

* Adjusted for sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding and maternal age.

† IgE-antibodies ($\geq 0.35\text{K U}_N/\text{L}$) to either inhalant, food, or cockroach allergens

Table 8. Odds Ratios and 95% confidence intervals for allergic diseases and sensitization associated with farm-related exposures after birth in children from Saint-Petersburg, Russia.

Animal contact for child	OR adjusted*						
	Physician-diagnosed asthma ever	Asthma current symptoms	Physician-diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician-diagnosed atopic eczema ever	Atopic eczema current symptoms	IgE antibodies to any of the allergens tested†
Any farm animal	1.25 (0.73-2.13)	1.08 (0.70-1.66)	0.84 (0.42-1.70)	0.76 (0.43-1.34)	1.25 (0.92-1.71)	0.98 (0.71-1.36)	1.05 (0.67-1.64)
Cow	1.17 (0.55-2.47)	1.50 (0.89-2.54)	0.49 (0.14-1.65)	0.84 (0.39-1.81)	1.48 (0.99-2.23)	1.09 (0.70-1.70)	1.39 (0.78-2.48)
Hens	0.92 (0.47-1.81)	1.02 (0.62-1.70)	0.90 (0.40-1.99)	0.66 (0.33-1.34)	1.28 (0.90-1.83)	0.90 (0.61-1.34)	1.15 (0.70-1.91)
Horses	1.16 (0.44-3.07)	1.50 (0.77-2.95)	1.40 (0.51-3.81)	0.18 (0.02-1.35)	1.14 (0.64-2.02)	0.87 (0.47-1.62)	1.13 (0.49-2.61)
Pigs	1.55 (0.69-3.45)	2.08 (1.17-3.70)	1.05 (0.35-3.17)	0.80 (0.31-2.07)	1.30 (0.78-2.14)	0.87 (0.50-1.53)	1.35 (0.70-2.57)
Rabbits	1.01 (0.41-2.45)	1.05 (0.53-2.07)	0.96 (0.32-2.84)	1.08 (0.47-2.47)	0.99 (0.59-1.66)	0.93 (0.55-1.58)	0.84 (0.41-1.71)
Goats	1.94 (0.98-3.82)	1.26 (0.68-2.33)	0.89 (0.31-2.62)	1.03 (0.45-2.35)	1.12 (0.68-1.84)	0.79 (0.45-1.37)	0.97 (0.49-1.92)
Sheep	1.56 (0.58-4.20)	1.64 (0.79-3.43)	1.25 (0.36-4.35)	0.52 (0.12-2.25)	0.82 (0.40-1.66)	0.58 (0.26-1.32)	0.56 (0.18-1.73)

* Adjusted for sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding and maternal age.

† IgE-antibodies ($\geq 0.35\text{K U}_N/\text{L}$) to either inhalant, food, or cockroach allergens.

Table 9. Odds Ratios and 95% confidence intervals for allergic diseases and sensitization in association with farm-related diet in children from Saint-Petersburg, Russia.

Consumption once a week or more	OR adjusted*						
	Physician-diagnosed asthma ever	Asthma current symptoms	Physician-diagnosed allergic rhinitis ever	Allergic rhinitis current symptoms	Physician-diagnosed atopic eczema ever	Atopic eczema current symptoms	IgE antibodies to any of the allergens tested [†]
Farm-milk	0.49 (0.17-1.39)	0.79 (0.40-1.58)	1.23 (0.50-3.03)	0.70 (0.29-1.67)	0.38 (0.20-0.70)	0.71 (0.43-1.19)	0.60 (0.27-1.31)
Farm vegetables and fruits	0.74 (0.46-1.21)	1.02 (0.70-1.48)	0.94 (0.53-1.67)	1.12 (0.71-1.75)	1.04 (0.80-1.36)	1.06 (0.80-1.40)	0.89 (0.60-1.33)
Fermented food	0.22 (0.03-1.66)	0.70 (0.27-1.84)	1.21 (0.35-4.18)	0.96 (0.33-2.77)	0.67 (0.34-1.35)	0.67 (0.33-1.38)	1.13 (0.46-2.80)

* Adjusted for sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding, maternal age.

[†] IgE antibodies ($\geq 0.35\text{kU}_A/L$) to either inhalant, food, or cockroach allergens.

6 DISCUSSION

6.1 INTERPRITATION AND IMPLEMENTATION

Part 1

6.1.1 Paper I: EBV seropositivity and allergic diseases

In the BAMSE study the prevalence of EBV infection among children at age four was around 50%. These data are in line with the results of corresponding studies from Sweden and other countries [18, 22, 207-210] (Figure 11).

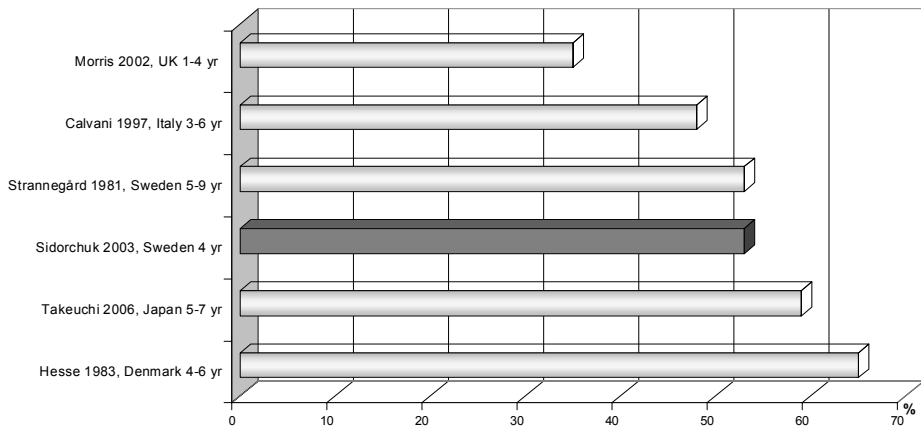


Figure 11. Seroprevalence to EBV in healthy children of preschool age from West European studies.

In our study neither a negative association previously seen in the younger age group nor a positive association reported for older children were reproduced. In light of the previously reported findings [19-21, 195-197] it is possible that primarily a long-standing, chronic EBV infection or primary infection at an older age may trigger allergy. Therefore, a positive association between EBV and allergy in older children, as described both by Strannegård [22] and Calvani [18], is not necessarily contradicted by our results, neither is the protective effect by EBV on sensitization of young children found by Nilsson [190]. Not all allergic diseases have developed at four years of age and further follow-up studies of the BAMSE cohort would be of value.

Two factors generally related to the development of allergic conditions - maternal smoking and young maternal age - were positively related to EBV seropositivity.

Several reasons may serve as an explanation for the findings, including that smoking mothers more frequently are infected with EBV, or that they shed more EBV in saliva, thereby becoming more contagious for their children. Since most adults are EBV seropositive, the first explanation is less likely. EBV replicates in the oropharyngeal epithelial cells during their terminal stages of differentiation [211, 212] and it has been reported that the mucosa of smokers tends to show a “higher” degree of terminal differentiation [213]. EBV shedding in saliva of HIV-infected persons has also been reported to be more frequent in smokers [214]. Being associated with EBV seroprevalence smoking could confound the association between EBV and allergy.

In children whose mothers were 25 years of age or younger, IgG-antibodies to EBV were more prevalent compared to children from older mothers. Increased shedding with more recent primary infection could be an explanation. This also makes mother's age a potential confounder that needs to be accounted for in analyses of EBV and allergy as there is evidence suggesting that younger mothers are more likely to have children who develop wheezing illnesses in early life [215].

6.1.2 Paper II: CMV seropositivity, interaction with EBV and allergic diseases

The prevalence of CMV seropositivity was 46% among the BAMSE children at age four, in line with previous epidemiological studies in developed countries [216, 217]. Our study does not support the hypothesis that previous CMV infection plays an important role in the pathogenesis bronchial asthma, allergic rhinitis or atopic dermatitis in children. However, in the absence of EBV infection, CMV infection may be related to sensitization to airborne and food allergens.

A positive association between young maternal age and seroprevalence to CMV may possibly be explained by an increase of CMV shedding related to more recent primary infections. The increase in primary infection of CMV in teenagers and young adults in Western countries, including Sweden, supports this assumption [218, 219]. A strong correlation between positive cervical cultures for CMV and young age was found among seropositive pregnant women [220]. Therefore, age-related effects may contribute to a productive CMV infection in mothers during pregnancy. This makes the age of mothers a potential confounder that needs to be accounted for in analyses of CMV and allergy [215].

In our study CMV infection was more prevalent in those who were breastfed during 4 months and more compared to other children. Prolonged excretion of virus in breast milk and in other body fluids is characteristic following CMV infection and plays an important role in transmission. Recent epidemiological studies have shown that the transmission rate of CMV infection from seropositive mothers to preterm infants by breastfeeding is 14-44% [221]. Some studies indicate that exclusive breastfeeding for longer than 4 months may delay the onset or protect against development of respiratory allergy in early life, but the relation between breastfeeding and the development of atopic dermatitis is still controversial [141, 142]. Isolauri et al. [222] found that

breastfeeding may maintain allergic symptoms in sensitized infants. A role of CMV infection via breastfeeding in this context deserves further attention.

The negative relation with size of the living area observed for children with CMV positive and double serostatus possibly result from a lack of close or prolonged contact with playmates who are excreting virus due to “non crowded” living conditions.

6.1.3 Paper III: RADUGA - environmental and lifestyle factors

The prevalence of allergy outcomes and sensitization found in our study is in line with data on allergy prevalence in Russian children revealed in the ISAAC Phase I and III [1, 23] and comparable with data from corresponding studies recently performed in Western Europe [9, 98, 107, 206]. Despite some inconsistencies in recent studies [23, 32-36, 39-42], there seems to be a tendency to increasing prevalence of allergic manifestations in children from Eastern European countries, including Russia. It has been suggested that the economic and social reforms, such as in Russia in recent decades, resulted in so-called “Westernization” of the country that was accompanied by rapid environmental and lifestyle changes. An increase in cigarette smoking, decrease in family size, changes in the dietary pattern, widespread use of antibiotics, etc., and, as a result a decline in certain childhood infections or a more general lack of exposure to infectious agents yearly in life, all may contribute to explaining an increase seen in allergic morbidity [33, 104, 112, 223, 224]. Another explanation may be related to the recent progress in diagnostic procedures, medical care and prophylactic practice. It has been shown that the diagnostic approaches and even diagnostic labeling may differ substantially between countries causing an underdiagnosis in some areas and, therefore, resulting in spurious geographical differences [25]. It appears that "asthma" diagnoses have been used less frequently in the Eastern European countries compared to Western Europe, since other respiratory diagnoses (bronchitis of various types) were used in the majority of children with respiratory symptoms. This is also supported by the differences seen in prevalence of allergic outcomes revealed in RADUGA using international methodology and data of official statistics from Saint-Petersburg (data from the annual report of the Saint-Petersburg Health Care Committee, 2001).

Another similarity between our findings and the results from epidemiological studies on allergy across the world is related to the role of various risk factors. Unsurprisingly, factors highlighted in other epidemiological studies on allergy [92, 162], such as migration from other countries [132, 134, 135], parental allergic history [82-86], and male sex [92-96, 98] were related to the development of allergic diseases in young children in Saint-Petersburg.

Some indoor exposures, such as ETS and wood smoke, were associated with allergic diseases, particularly asthma and rhinitis, in line to previous findings [104-106, 109, 110, 171-174]. Parental awareness of the detrimental effects of passive smoking on development of allergic disease in children appears to be low in Russia, considering the wide spread exposure. At the same time, our findings underscore the role of ETS in

enhancing the allergy development and, therefore, confirm the great importance of improving tobacco control practices in Russia.

6.1.4 Paper IV: RADUGA - farm-related factors

To our knowledge, RADUGA is the first study ascertaining the effect of farm-related exposures on development of allergic diseases among urban children. In Russia, farm-related activities as well as summerhouse ownership and consumption of self-grown vegetables and fruits are all rather prevalent, also among urban residents. Moreover, a high prevalence of farm milk consumption as well as of fermented food, frequently seen in children across Eastern Europe and Russia [225, 226], may be of specific interest for allergy development. It is important to note that in our study group there were no families constantly living in rural areas or reporting farming as current occupation, which indicates that primarily short-term, occasional exposure to farm environments occurred.

Our results apparently contradict the concept of a protective effect of farming environment on development of allergic diseases in children. The prenatal protective effect seen in children whose mothers were working at a farm during pregnancy may be attributable to the high level of microbial exposure associated with intensive and long-term contacts to livestock [64]. This was also supported by dose-response relations between the number of farm animal species the mother had contact with during pregnancy and upregulation of the genes for receptors of the innate immune system [64]. However, specific farm-related prenatal determinants of the innate immune response as well as an appropriate timing when the modulation of the immune response occurred remain unknown.

The effect of postnatal exposure to farm environment appears complex. The protective effect seen in a number of European studies on farm children has also been attributed to specific microbial compounds, i.e. child's exposure to endotoxin and extracellular polysaccharide. However, the same exposure can provoke chronic airway inflammation and airway hyperresponsiveness, particularly among professional pig farmers [227-231], and enhance risk of wheezing among infants with a parental history of atopy if exposure occurred in early childhood [232-234]. The timing of exposure may be of importance since the influence of farm environment on development of allergic diseases seems to vary by age, switching the protective effect of exposure in infants to detrimental effects if occurring later in life [26, 164, 170].

In RADUGA a regular exposure to livestock, particularly to pigs, during childhood was associated with current asthma symptoms, which seems to contradict results of European studies on farm children [65, 164], but confirm the results from studies performed outside Europe [172, 235-237]. The discrepancy may potentially indicate a different pattern and intensity of exposure due to different farm practices and routines. Causal relationships as well as the importance of age of exposure might only be possible to identify in longitudinal studies [64].

In our study, consumption of farm milk tended to be inversely associated with physician-diagnosed eczema, which confirms previous reports [8]. It is plausible that the composition of intestinal flora influences the development of the immune system [225, 226]. The inverse associations between farm milk and allergic conditions also support the idea that dietary interventions might be involved in primary prevention of allergy.

6.2 METHODOLOGICAL CONSIDERATIONS

In this thesis two different types of study design are used. Papers **I** and **II** were based on the results from a prospective birth cohort study, while papers **III** and **IV** dealt with the results of a cross-sectional questionnaire-based study. In a study with a prospective birth cohort design where data on exposures are obtained before the children have symptoms of allergic disease, the results are less subject to bias compared to study with a cross-sectional design and retrospective collection of exposure data [91].

Information bias

Misclassification of exposure.

In *BAMSE* the exposure information was collected repeatedly at certain time points to enhance precision. The questionnaire information on risk factors was obtained before the children developed allergic symptoms to minimize the risk of disease-related misclassification. Most exposure data, used in our analyses were collected when the children were on average 2 months old, thus prior to onset of disease. The exposure questionnaire was in 86% of the children completed by both parents together. Still, there is always a risk for methodological problems, such as underreporting of exposure, particularly in families with heredity, or denial of symptoms that could be related to exposure [238].

RADUGA did not include objective measurements of exposures. However, low parental awareness of detrimental effect of ETS, as seen in our study group, more likely results in nondifferential misclassification of exposure, which would lead to a weakening of any associations. Furthermore, most of the exposures to farm environment are not generally recognized as risk factors for development of allergy in children and, therefore, might also be recalled with low accuracy resulting in nondifferential misclassification.

Misclassification of outcome

The parentally reported questionnaire-based information on doctor's diagnosed allergic diseases and current symptoms may result in recall bias that is more likely to be differential. However, the *BAMSE* questionnaire was designed to aid accurate repeated recall. IgE-sensitization was defined by using highly precise laboratory techniques.

In the *RADUGA* study the same approach was used to reduce misclassification of self-reported outcomes. The questionnaire was carefully framed and based on previously validated *BAMSE* and *PARSIFAL* questionnaires. A clinical examination, included in

RADUGA, allowed to specify the information of doctor's diagnosed allergic diseases and current symptoms. However, the clinical examination was performed one year later than the questionnaire survey and could not verify the issue of "current" symptoms. Similar to BAMSE, IgE-sensitization was defined by using highly precise laboratory techniques.

Selection bias

The *BAMSE study* was able to maintain high follow-up rate that was mostly a result of having a study team highly successful in keeping contact with families and tracking down those who moved away as well as due to the fact that this is a single-centre study. The children with missing data seem to differ somewhat from those included in the BAMSE analyses, e.g. in socio-economic status and exposure to tobacco smoke, which introduces some selection bias. However, the number of missing children is relatively small and no difference was seen in sex and parental allergic diseases. Non-response is therefore unlikely to influence our interpretation to any major extent [92].

There is a potential risk of selection bias in *RADUGA* if parents of sensitive children are more prone to choose private daycare. More than 60% of the children in Saint-Petersburg attend public day care [239] and our findings should be generalizable to this group. The size of the subgroup with blood samples and subsequent immunological analysis was relatively small resulting from a high non-response. Furthermore, socio-economic characteristics and prevalence of allergic diseases differed between children providing blood samples and those not sampled indicating that parental awareness of the child's allergic conditions affected the selection. The prevalence of sensitization to any IgE-antibodies observed in *RADUGA* was 30.2%. A "true", recalculated prevalence of sensitization was found of about 28.4 – 28.9%. Based on the new data it was concluded that the prevalence of atopic sensitization observed in our study is somewhat overestimated. However, there is no substantial difference between observed and a "true" prevalence of sensitization.

Confounding

After testing several models only parental allergic disease, maternal age, maternal smoking during pregnancy, gender of the child, parental educational level and duration of breastfeeding were identified as confounders in *EBV/CMV studies*. However, the possibility that unmeasured confounding may have contributed to the observed associations can not be excluded.

The variables adjusted for in the analyses of the *RADUGA study*, included sex, age of the child, district, mother's and father's reported asthma or rhinoconjunctivitis, educational level of the parents, maternal smoking during pregnancy, current environmental smoking, number of older siblings, duration of breastfeeding and maternal age. Variables were included in the model based on two criteria. Either they changed the risk estimate by 15% or more when included or they were generally recognized risk factors for allergy in children. Most variables fulfilled both criteria.

7. CONCLUSIONS

- The study does not support the hypothesis that EBV or CMV infections play an important role in the pathogenesis of allergic diseases in Swedish children at 4 years of age. However, in the absence of EBV infection, CMV infection may be related to sensitization to airborne and food allergens.
- An antagonistic interaction was suggested between CMV and EBV in relation to sensitization. These two viruses may theoretically counteract the effect of each other on the immune system, which could partly explain the heterogeneous findings in different studies.
- The associations between EBV/CMV infections and risk factors for allergy in children, such as young maternal age and maternal smoking, make these factors potential confounders that need to be controlled in analyses of EBV/CMV and allergy.
- The occurrence of allergic diseases and sensitization among Russian children from Saint-Petersburg appears similar to the prevalence among children in Northern and Western Europe.
- A number of indoor environmental exposures appear to be of importance for development of allergic diseases in children of Saint-Petersburg, Russia, such as environmental tobacco smoke and wood smoke. Other factors generally known to be related to allergic conditions, such as migration from other countries and parental allergy, also contribute to the occurrence of allergic manifestations.
- Exposure to farm-related environments, particularly contact of the mother with certain livestock, appears to be associated with allergic conditions among children in Saint-Petersburg. On the other hand, regular consumption of farm milk tended to be protective, confirming earlier evidence.
- There is a substantial potential for prevention of allergy among children in Saint-Petersburg, e.g. by reducing smoking among women of childbearing ages.

8. RUSSIAN SUMMARY

Рост аллергической заболеваемости у детей, особенно заметный в экономически развитых странах, является объектом международного внимания. Одним из факторов, ответственных за увеличение числа аллергических заболеваний, предположительно является сокращение числа детских инфекций или общее снижение риска встречи с возбудителем инфекционных заболеваний в детском возрасте.

В последние годы в странах Восточной Европы и в России отмечался рост аллергической заболеваемости сходный с ранее наблюдавшимся в Западно-Европейских странах. Предполагаемым вкладом в увеличение числа аллергических заболеваний явилась так называемая «вестернизация» Восточно-Европейских стран, то есть переход к рыночной экономике, сопровождающийся изменениями в экономической и социальной сферах и повлекший за собой определенные изменения в образе жизни населения и состоянии окружающей среды. На сегодняшний момент ощущается недостаток достоверных эпидемиологических данных, полученных в ходе популяционных исследований, касающихся как уровней детской аллергической заболеваемости в Российской Федерации, так и роли ведущих факторов риска в возникновении и распространении данной патологии.

Первая часть диссертационной работы включает два исследования, посвященных изучению роли хронических инфекций, вызванных вирусом Эпштейна-Барра и Цитомераловирусом в возникновении и развитии аллергических заболеваний и сенсибилизации у шведских детей четырехлетнего возраста. В исследуемую группу был включен 2581 ребенок, ранее входивший в шведское проспективное когортное исследование (BAMSE) по изучению факторов риска развития аллергических заболеваний у детей. Данные о наличии аллергических заболеваний основывались на результатах анкетирования, проведенного в рамках BAMSE среди родителей детей 4-х летнего возраста. Данные о сенсибилизации к различным группам ингаляционных и пищевых аллергенов были получены в ходе проведения иммунологических анализов. Иммуноглобулин G (IgG) к вирусу Эпштейна-Барра был определен методом непрямой иммунофлюоресценции. Серологические реакции для определения IgG к Цитомераловирусу были проведены с использованием энзим-связанного иммуносорбентного анализа. IgG-антитела к вирусу Эпштейна-Барра и Цитомераловирусу выявлены в сыворотках 46% и 53% детей, соответственно. При этом, эксклюзивная серопозитивность к вирусу Эпштейна-Барра (при отсутствии антител к Цитомераловирусу) была определена у 26% детей. Сыворотки 20% детей, напротив, продемонстрировали наличие Ig G к Цитомераловирусу при отсутствии антител к вирусу Эпштейна-Барра. Результаты анализов 25% сывороток выявили наличие антител к обоим исследуемым вирусам. При изучении роли данных вирусов в возникновении и развитии аллергических заболеваний у детей раннего возраста, статистически значимых связей с аллергической патологией выявлено не было. Однако, в отношении сенсибилизации была выявлена связь с наличием антител к Цитомераловирусу

при отсутствии, у тех же детей, антител к вирусу Эпштейна-Барра. Помимо этого, в группе детей с признаками сенсibilизации статистически был выявлен антагонизм между исследуемыми вирусами.

Второй частью диссертационной работы явилось проведение Российско-Шведского эпидемиологического «РАДУГА» (Распространенность Аллергии в Дошкольных Учреждениях Города (Санкт-Петербурга)), с целью определения превалентности аллергических симптомов, врачебных диагнозов и признаков сенсibilизации в популяции детей дошкольного возраста в Санкт-Петербурге. Целью исследования также явилось выявление степени влияния различных факторов внешней среды и особенностей жизнедеятельности на развитие аллергических заболеваний у детей. Методологические подходы к процессу сбора данных, а так же процедура проведения проекта «РАДУГА» были основаны на опыты зарубежных эпидемиологических исследований, что позволило получить данные сравнимые с результатами международных исследований в области аллергических заболеваний. Среди предполагаемых факторов риска средовой природы особое внимание было уделено изучению роли неурбанизированной, сельской окружающей среды, а именно, факторов, относящихся к пребыванию в сельской местности, участию в сельскохозяйственной деятельности, в частности уходу за животными, на развитие аллергии у детей-жителей крупного города. Исследуемая группа включала 1702 детей дошкольного возраста, посещающих детские дошкольные образовательные учреждения в трех выбранных для проведения проекта районах Санкт-Петербурга. Поперечное эпидемиологическое исследование «РАДУГА», включало в себя анкетирование родителей с целью сбора информации о воздействии на ребенка в пре- и постнатальный период различных факторов внешней среды и особенностей жизненного уклада семьи, данные о состоянии здоровья ребенка, а также его аллергологический анамнез. Исследование также включало сбор анализов крови для последующего определение титра аллерген-специфического IgE в сыворотке крови к смесям ведущим ингаляционным и пищевым аллергенам. В ходе анкетирования был достигнут достаточно высокий уровень участия, который составил 85,1%. По результатам анкетирования аллергические заболевания были выявлены у 23% детей. Признаки сенсibilизации к одному или нескольким ингаляционным и пищевым аллергенам были выявлены у 30% детей. Среди исследуемых факторов средовой природы наиболее значимые ассоциации с наличием у ребенка бронхиальной астмы были установлены для так называемого «пассивного курения» и экспонированности ребенка к продуктам сгорания твердого топлива (дрова, уголь). Помимо связи с высокой заболеваемостью бронхиальной астмой, указанные средовые факторы риска явились статистически значимыми для развития заболеваемости у детей с наличием диагностированного аллергического ринита, а также симптомов астмы и ринита. В отношении сенсibilизации статистически достоверных ассоциаций с факторами риска внешней среды выявлено не было. У детей, подвергавшихся пре- или постнатально воздействию факторов риска, относящихся к пребыванию в сельской местности и уходу за животными, был выявлен рост аллергических заболеваний, при отсутствии изменений в превалентности сенсibilизации. Среди «сельских» факторов риска фактором, оказывающим превентивное действие на развития детских

аллергических заболеваний в данной группе, явилось регулярное употребление фермерского коровьего молока.

Основными выводами из приведенных выше исследований являются следующие. Наши результаты не подтверждают ранее сформулированную гипотезу о значимой роли хронических инфекций, вызванных вирусом Эпштейна-Барра и Цитомераловирусом в возникновении и развитии аллергических заболеваний или сенсibilизации у детей в возрасте 4-х лет. Однако, в отношении развития сенсibilизации данные вирусы демонстрируют статистически подтвержденный антагонизм, подчеркивая, тем самым, необходимость и важность изучения взаимодействий различных инфекционных агентов и влияния результатов подобного взаимодействия на деятельность иммунной системы ребенка. Проведенное Российско-Шведское эпидемиологическое исследование выявило высокие уровни аллергической заболеваемости у детей дошкольного возраста в Санкт-Петербурге, схожие с уровнями, ранее заявленными по результатам исследований, проведенных в Скандинавских странах и странах Западной Европы. Спектр факторов различной природы, оказывающих, по результатам исследования, влияние на развитие аллергической заболеваемости у детей Санкт-Петербурга, является достаточно близким к факторам, определенным в ряде международных и Западноевропейских проектах как «факторы риска развития аллергических заболеваний в раннем возрасте».

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