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Microbial agents, allergens and atopic diseases - contributions to the PARSIFAL study

Microbiële componenten, allergenen en allergische aandoeningen – bijdragen aan de PARSIFAL studie

(met een samenvatting in het Nederlands)

Proefschrift

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Iedereen is tevreden, voldaan gebleven
met de sinistere uitspraken van gehaaste kapitalisten
en systematische vrouwen.
Ik wil met veel dingen spreken
en ik wil deze planeet niet verlaten
zonder uiteindelijk te begrijpen
wat ik heb gevonden,
zonder deze zaak te hebben opgelost,
mensen zijn niet genoeg.

Dus, mijne heren, ga ik converseren met een paard;
Laat de dichteres mij excuseren
en de professor mij verlof geven.
De hele week zal ik bezig zijn.

Pablo Neruda

CONTENTS

CHAPTER 1

Introduction

Dust – from measure of smallness to the infinity of the infinitesimal

Once, not so long ago, the smallest thing known was dust itself. Since the discovery of the microscope, however, dust has been redefined and is now seen as a highly diverse particulate [1]. We are now able to measure very specific components in house dust, like pet and mite allergens and microbial agents, which are the scope of this thesis.

The book 'Dust – the history of the small and the invisible' by Amato describes how we, in the course of Western civilization, learned to described smaller and smaller particles of the natural world, which forced humans to recognize 'the immensity and might of the small' or, in Amato's words, 'the infinity of the infinitesimal'.

In the course of the 19th and the beginning of the 20th century exposure to dust and dirt was highly reduced, since sanitarians asserted a relationship between dust and disease [2-4], and new inventions made cleaning easier. Doctors and nurses began to wash their hands between patients, governments implemented sewage systems and ordered delousing, pasteurizing and sterilizing and individuals started vacuum cleaning [1, 5, 6]. Infectious diseases were strongly reduced by this increase in hygiene. However, other disorders than those transmitted by microorganisms, such as allergy and asthma, showed a rising prevalence during the last decades [7-9]. It has been suggested that something, perhaps in dust, may lack from our societal affluence that has the capacity to protect against the development of atopic diseases [9]. Much attention during the last years has been devoted to the 'hygiene hypothesis', which proposes that certain medical interventions, like use of antibiotics and vaccinations and/or reduced exposure to microbes have resulted in the increase in atopic diseases.

After providing definitions of atopy and asthma, the epidemiological and immunological bases of the hygiene hypothesis are described in this chapter. In addition, microbial agents and allergens, which we measured in house dust, are described, followed by a description of the PARSIFAL project and aims of this thesis.

Atopy and asthma

Asthma is a chronic inflammatory condition of the lung airways, of which the cause is incompletely understood. In susceptible individuals this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and/or in the early morning. These symptoms are usually associated with variable airflow limitation. In asthma, airways are often hyperresponsive to many different stimuli, such as allergens, cigarette smoke, cold air or viral infections [10].

The term 'atopy' is used to describe those individuals who readily develop antibodies of IgE class against common allergens in the environment. Such antibodies are present in 30-40% of the population. Most asthmatics (80%) are sensitized to at least one common allergen [11, 12]. It has been assumed that a direct association between allergen exposure and asthma exists, in which in first instance allergen exposure leads to allergic

sensitization in predisposed individuals, and continued allergen exposure leads to airway inflammation and asthma [13, 14]. However, results of studies on this issue are conflicting [15-19] and not all asthma cases can be attributed to atopy [10, 20].

HYGIENE HYPOTHESIS

Epidemiological basis

In 1989, Strachan reported an inverse relationship between hay fever and the number of children in the household in a cohort study of 17,414 British children. He suggested that declining family size, improvements in household amenities and higher standards of personal cleanliness had reduced the risk of cross infection in young families and that infections might prevent allergic diseases [21]. Since then, numerous other studies have shown an inverse relationship between number of older, and to a lesser extent, number of younger siblings and the occurrence of atopy, hay fever and eczema in children and adults, as reviewed by Karmaus and Botezan [22]. For asthma however, the relationship appears to be more complex, because several studies have not been able to confirm such an inverse relationship for asthma [22]. Also for daycare attendance, another supposed marker of exposure to infectious agents, inverse relations with atopy, hay fever and asthma have been observed [23-26].

Subsequent studies to identify infections as the explanatory protective factor for the sibling and daycare attendance effect have shown conflicting results [27]. Some studies found no effect of common infectious diseases in early life [28, 29], whereas others did find a protective effect [30, 31]. For example, a study among 1,314 German children followed from birth to the age of 7 years, suggested that repeated viral infections in early life, particularly common colds and infections of the Herpes type, reduced the risk of developing asthma in a dose-dependent manner [30]. Discrepant results from studies into the effects of infections might partly be due to differences in the way 'infections' have been assessed, e.g. by reports of viral infections or clinical assessments. Furthermore, the effects may depend on the timing of exposure: before of after the development of allergic sensitization [32]. In addition, markers of infections that are more easily spread under crowded and unhygienic conditions, do not only represent exposure to specific infectious agents, but also to higher levels of bacterial products in general, that may confer a protective effect [27]. This might explain the strong inverse relationships which have been observed between serological markers of previous infections with food-borne and oro-fecally transmitted microbes, such as hepatitis A, and the prevalence of atopy [33-35].

Another line of evidence for the hygiene hypothesis comes from geographic or regional differences in the prevalence of allergic conditions [9]. Two large international surveys, one in children [the International Study of Asthma and Allergy in Childhood

(ISAAC)] and the other in adults [the European Community Respiratory Health Survey (ECRHS)], showed considerable variation in asthma prevalence between countries and markedly higher prevalences in developed than in developing countries [36-38]. Interesting differences were also noted in a comparison of the prevalence of allergic diseases in Eastern and Western Germany after the German reunification in 1989. It was shown that in genetically similar individuals, the prevalence of allergic diseases, and atopy in particular, were significantly higher among school children living in the former West as compared to children in former East Germany [39]. These observations illustrate the importance of environmental factors, associated with increasing affluence, in the development of allergic diseases [9].

Epidemiological studies in children from farmers

Since the late 1990s, a series of epidemiological studies consistently showed a reduced risk of hay fever and atopy in children from farming families compared with their peers from non-farming families [40-42]. A protective effect on asthma was not observed in all of these studies [43]. Actually, first observations of the lower prevalence of hay fever among farmers date back to 1873, when Blackley described hay fever as 'an aristocratic disease' [44]. The European Community Respiratory Health Survey showed that the increase in atopy and allergic rhinitis was not observed in individuals who were exposed to a farming environment in childhood [45] and three other studies showed that the protective effect of a farm childhood also persisted into adulthood [46-48]. In contrast to the findings of European farm studies, a study in New Zealand showed that farm children had more hay fever and asthma, but not more atopy, than children not living on a farm [49].

The protective effect of living on a farm might be due to larger family size, more pets, less maternal smoking, more dampness and different dietary habits [50]. However, none of these factors have been shown to play a major role. By contrast, contact to livestock has been found to explain much of the inverse association between farm environment and atopy [40, 42]. Since other studies showed that animal farmers are exposed to high levels of fungal and bacterial products, including endotoxin [51, 52] with known immunomodulatory effects [53-55], it was suggested these agents may be responsible for the lower prevalence of atopic diseases among farm children. This was investigated in the ALEX (being an acronym for 'allergy and endotoxin') study, a cross-sectional study of more than 800 rural school-age children in Switzerland, Austria and southern Germany. The prevalence of sensitization was 17% in farm children versus 24% in non-farm children, for hay fever and atopic asthma these percentages were 4 versus 11% and 3 versus 6% respectively [56]. In addition, the study showed elevated levels of endotoxin in the homes of farm children and children with regular contact to livestock as compared to non-farm children without animal contacts [57]. Additional analyses showed an inverse relation between mattress levels of endotoxin and the occurrence of sensitization, hay fever and

atopic asthma [56]. Inverse relations between those atopic diseases and first-year exposure to farm characteristics, i.e. exposure to stables and drinking farm milk, were observed as well [58]. The effect of endotoxin was observed in addition to and independent of the effect of first-year exposure to farm characteristics, and was not restricted to farm homes [56].

Epidemiological studies in children from anthroposophic families

In 1999, a low prevalence of allergic diseases was observed in children from families with an anthroposophic lifestyle [59]. The school of anthroposophy (Greek: wisdom about man) was founded by Rudolf Steiner [60] and has been applied to education (Steiner schools, in Dutch 'Vrije scholen'), medicine, art, architecture, agriculture (biodynamic farming) and diet [61]. Anthroposophical doctors restrict the use of antibiotics, antipyretics and vaccinations because illness is viewed as a 'positive and necessary factor in the destiny of the child' and treatment of the illness is believed to interfere with this process [62]. The study by Alm et al. included 295 children from Steiner schools, whose parents predominantly adhere to an anthroposophic lifestyle, and 380 children from two neighboring schools in Sweden. The prevalence of atopy was 24% in children from Steiner schools versus 34% in the other children, for wheeze these percentages were 3% versus 8%. There was an inverse relation between the number of anthroposophic lifestyle features, e.g. restricted use of vaccinations and antibiotics and consumption of biodynamic food, and risk of atopy [59].

Another study in children from Steiner schools was performed in New Zealand, but this study did not include children from other schools as a reference [63]. It showed a retrospectively association between antibiotics use and an increased risk of asthma, which could explain the lower prevalence of wheeze in children from Steiner schools, as observed by Alm et al. [59]. However, results from recently conducted prospective studies suggest that the association between antibiotics use in early childhood and asthma, as observed in several cross-sectional studies, including the study by Wickens et al., is not a causal relationship; frequent use of antibiotics may rather be a marker of an increased risk of being diagnosed with asthma later in childhood [64]. Alternatively, children who are predisposed to develop asthma might also be more susceptible to infectious diseases requiring antibiotics in early childhood. The effect of vaccinations on subsequent development of asthma and atopy also remains a matter of controversy; some studies suggested that immunization might increase the risk of atopic disease, whereas other studies found no association or have even reported a protective effect against atopy [65].

Alm et al. further explored the possible mechanisms behind the observed protective effect of an anthroposophic lifestyle by comparing the gut microflora from young (< 2 years old) children of parents with this lifestyle and parents with a conventional lifestyle, and it was concluded that the gut microflora was influenced by several lifestyle characteristics, like vegetarian diet and antibiotics use [66]. As the intestinal flora is considered to have an impact on the development of the immune system [67], and differences

in microflora between allergic and non-allergic children have been observed [68], it has been inferred that differences in gut microflora could explain the observed lower risk of atopic diseases in Steiner children.

Immunological basis

The immunological basis of the hygiene hypothesis is still controversial, but it has been suggested that reduced microbial exposure results in an imbalance between two types of T helper cells, which are characterized by the signaling proteins (cytokines) they produce [69]. It has been well documented that the type 2 T helper (Th2) cells, characterized by their production of Il-4, Il-5 and Il-13 cytokines, play a critical role in the pathogenesis and maintenance of allergy, by inducing B cells to produce IgE-antibodies. Type 1 T helper (Th1) cells producing INF-γ, on the other hand, play a role in elimination of many types of bacteria and viruses [70-72]. Children are born with strong type 2 responses but mature their type 1 responses in their first years of life under environmental influence, mainly that of common childhood infections [73]. Therefore, less exposure to infectious agents, but maybe also to microorganisms in general, may result in reduced immune deviation from a Th2 (atopic) to a Th1 (normal) phenotype.

A more recent explanation is that less exposure to microbial agents results in reduced immune suppression by regulatory T (Treg) cells, which would result in both higher Th1 and Th2 responses. Although evidence for the latter explanation is scarce, it is likely that both mechanisms play a role [69]. The influence of environmental factors on the network of T cells, including Th0, Th1, Th2 and Treg, was nicely illustrated in a study in eastern and western Germany; individuals who grew up in eastern Germany had a marked bias towards Th0 cells, whereas children of western Germany showed Th2 polarization [74], although it is unknown which factors accounted for this effect. Major factors involved in the current concept of the hygiene hypothesis are summarized in figure 1.

ENDOTOXIN, ß(1,3)-GLUCANS AND FUNGAL EXTRACELLULAR POLYSACCHARIDES (EPS)

It was a provocative idea to consider endotoxin as potentially protective against the development of asthma and allergies, because it has been known for years as a potent inducer of adverse health effects in industrial workers and farmers, who are exposed chronically to high concentrations and who develop severe respiratory diseases [27]. Endotoxin is a strong pro-inflammatory agent from the cell wall of gram-negative bacteria and when inhaled in high quantities, it elicits acute clinical effects like bronchoconstriction and fever [75]. Furthermore, patients with asthma are hypersensitive to endotoxin compared to non-asthmatic individuals, and the severity of the disease is positively correlated with the

amount of endotoxin in their home [76]. Endotoxin is a potent inducer of Il-12 and IFN-γ, which are key regulators of Th1-type immune development and therefore, it is tempting to hypothesize that Th2 cytokine production is inhibited by endotoxin [77]. This might explain why endotoxin, apart from harmful effects, could also be potentially protective, if the exposure happens to occur very early in life, and thereby induces immune deviation to a non-atopic phenotype [27].

Furthermore, endotoxin might rather protect against atopy than against asthma; it has been suggested that endotoxin may prevent the primary causation of allergic asthma, but might be a primary and secondary cause of non-allergic asthma [78]. This view is supported by a study of Portengen et al., who showed that exposure to endotoxin appeared to protect from sensitization to common allergens in adult pig farmers [79], whereas analyses in the same population showed that high endotoxin exposure was associated with respiratory symptoms [80]. In a Norwegian study among 2,169 farmers, most asthmatic subjects were non-atopic (80%) and estimated air exposure levels to endotoxins, fungal spores and ammonia were positively associated with the non-atopic asthma prevalence and negatively with the atopic asthma prevalence [81]. However, further research on potential harmful and/or protective effects of endotoxin is needed, by follow-up of farm children and/or young farmers, focusing on timing and dose of endotoxin exposure and characterization of asthma phenotypes.

Protective effects of endotoxin against atopy in other than farming populations have been observed as well. The first study that showed that endotoxin exposure early in life might protect against atopy by enhancing type 1 immunity, was performed by Gereda et al., in a population of 61 children with wheeze. They found that the homes of allergen-sensitized children contained significantly lower endotoxin levels in house dust than those of non-sensitized children. Importantly, they also showed that endotoxin levels in house dust correlated with proportions of Th1 cells, but not with Th2 cells in blood [82]. In a cross-sectional study among school-age children in Germany, exposure to higher levels of house dust endotoxin was associated with a lower prevalence of allergic sensitization [83]. In a case-control selection of 350 adults participating in the German part of the ECRHS, it was shown that current exposure to higher levels of house dust endotoxin was associated with a decreased risk of allergic sensitization in adults [84]. However, an inverse association between endotoxin and atopy could not be confirmed in a prospective study of 2000 infants [85], and another prospective study showed that exposure to high concentrations of house dust endotoxin was associated with an increased risk for wheezing, although this risk rapidly decreased over time [86]. It has been suggested that the effect of endotoxin may vary with the type, timing, and dose of concomitant allergen exposure, in addition to genetic background of the children [85].

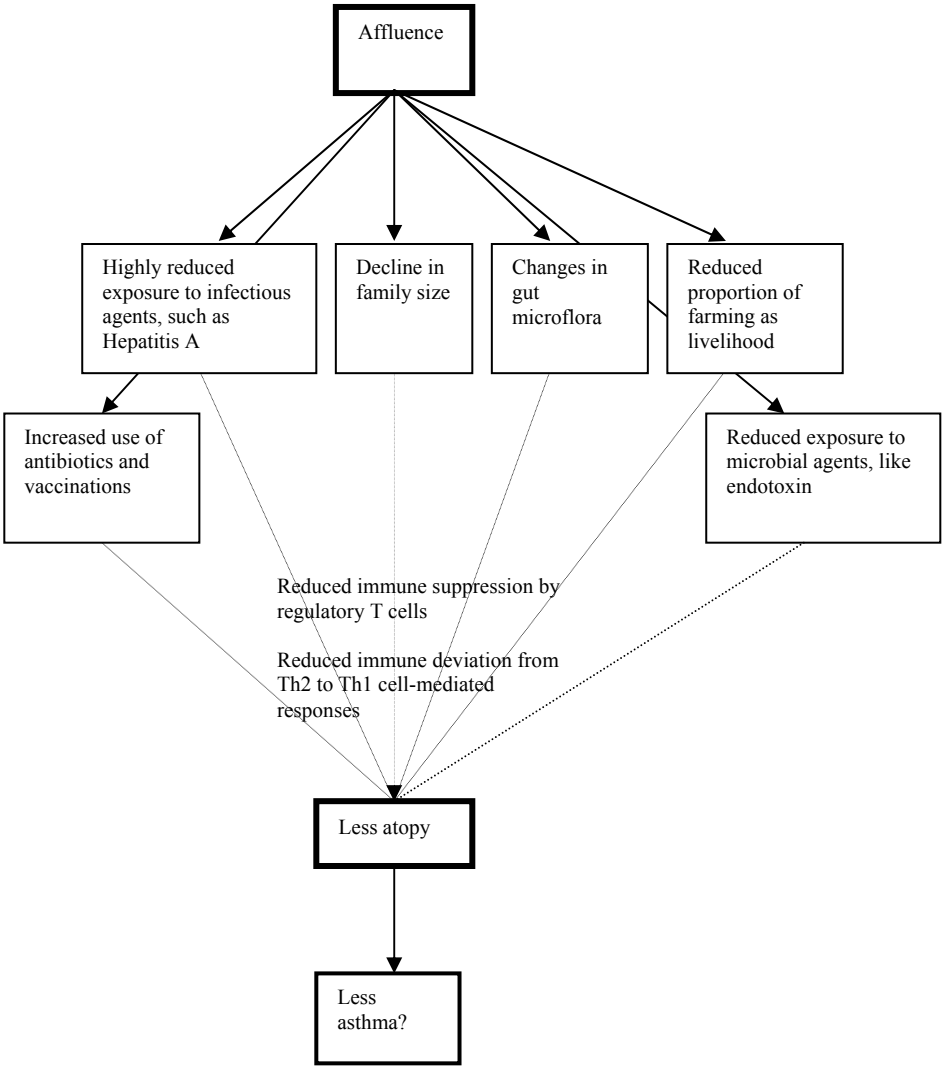

Figure 1. Major factors suggested to be involved in the current concept of the hygiene hypothesis. Adapted from von Hertzen and Haahtela [9].

Most research regarding potentially protective effects of microbial exposure has focused on endotoxin so far, although it has been suggested that endotoxin, for example in studies among farmers, might only be a marker of exposure to other microbial agents [57]. It is likely that other microbial agents, like mould components, may affect immune responses in a similar way as endotoxin [57, 87]. ß(1,3)-glucans, cell wall constituents of most fungi, for example, have strong immunomodulatory effects [55, 88-92]. Although dampness and moulds have been associated with an increased risk of asthma [93-95], most studies were cross-sectional and therefore it is not clear whether moulds cause or only exacerbate preexisting respiratory symptoms such as asthma [87]. Mould components thus may have both harmful and protective effects on atopic diseases, depending e.g. on timing and dose of exposure, as suggested for endotoxin. It has been shown that ß(1,3)-glucans and fungal extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp.* are associated with total culturable fungi in house dust, and thus may be good markers for indoor fungal exposure [96].

PET AND MITE ALLERGENS

An alternative explanation for the rising prevalence of atopic disease might be an increased exposure to house dust mite allergens or other allergens [97]; homes are better insulated and less ventilated nowadays, which might stimulate mite growth, but there is no evidence that indoor mite allergen levels have increased during the last decades. In addition, people spend more than 95% of their time indoors, while this figure was much lower in the past [98]. The lower prevalence of atopic diseases in farm children and children of anthroposophic families could potentially be attributed to a lower exposure to pet and mite allergens.

The importance of house dust mites in allergies has since long been recognized [99, 100]. Several studies have shown a dose-response relationship between house dust mite exposure and sensitization to house dust mites [16, 101-103]. Furthermore, it is well established that dust mite allergen exposure is a secondary cause of asthma; it can trigger asthma attacks in sensitized asthmatic subjects and prolonged exposure can lead to the persistence of symptoms [20]. However, it is not clear whether house dust mite exposure is a primary cause of asthma [20].

The evidence for a causal relationship between pet allergens and asthma is weaker than for mite allergens, although studies have shown that exposure to cat and dog allergens might be a more important cause of sensitization and thereby, asthma, in areas where house dust mites are not abundant, such as high altitudes or areas with a dry climate, such as Northern Sweden [104]. However, the topic of associations between pet exposure, sensitization and asthma is confusing, because many conflicting and intriguing findings have been published recently [105]; cat exposure increases [106] or decreases [107], and dog exposure decreases [108] or has no effect [109] on the risk of sensitization; asthma is negatively

[109] or positively [110] associated with dog exposure, and cat exposure can increase [17] or decrease the risk of asthma [107]. The complexity of the issue may partly be related to selection effects; people do not have pets (anymore) because of allergies, which leads to wrong conclusions when current pet ownership is related to current symptoms. Another reason for the complexity might be that cat and dog allergens are ubiquitous in society and may affect sensitization regardless of pet ownership [111]. It has been speculated that the possible protective effects of pets could be attributed to higher endotoxin levels in homes where these animals are kept [112]. However, a longitudinal study showed that high levels of cat allergen and having a dog in the home decreased the risk of wheezing, independent of endotoxin levels in house dust [86].

PARSIFAL STUDY AND AIM OF THIS THESIS

In 2000, the cross-sectional PARSIFAL (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) study was started. The goal of PARSIFAL was to explore environmental and lifestyle factors characteristic of farming and anthroposophic populations that may protect against the development of atopic diseases in children. The study was performed in Austria, Germany, the Netherlands, Sweden, and Switzerland. A total of 21,905 children aged 5-13 years from farmers, families with an anthroposophic lifestyle, and respective reference families were invited to participate. Children with an anthroposophic lifestyle were recruited through Rudolf Steiner schools. The reference children were selected such that they lived in the neighborhood of farm and Steiner school children, but did not actually live on a farm or did not attend a Rudolf Steiner school, respectively. A total of 14,893 children actually participated. Information regarding atopic diseases, home and lifestyle characteristics was assessed through a questionnaire administered to the parents of the participating child. Objective measurements of atopic sensitization were obtained by immunoglobulin E (IgE) serology in a sub-sample of 4,039 children, whose parents gave consent to blood sampling. In addition, house dust was collected from homes of families who gave consent to dust sampling, in two different ways:

- With ALK devices, by fieldworkers during a home visit
- With nylon sample socks, by parents, who received and returned socks by mail

ALK sampling nozzles have been widely used in epidemiological studies, assessing allergens or endotoxin in samples of settled house dust. Dust sampling with nylon socks is less commonly applied, although it has important logistical advantages; it enables dust sampling by parents instead of fieldworkers, because, in contrast to ALK devices, socks can

be easily mailed to participants, who return socks with dust. This is particularly useful for large population studies, like the PARSIFAL study. To validate the sock collection method, dust was also collected by fieldworkers with ALK devices in a sub-sample of the PARSIFAL population (n=478). In addition, as an extra validation, fieldworkers also collected dust with nylon sample socks in a sub-sample of homes (n=114) during the same home visit as ALK dust samples were collected.

For ALK dust collection by fieldworkers, circa 100 children per country; 50 farm children, 25 children from Steiner schools and 12-13 children of each reference group, were selected randomly from the total population. For dust collection by parents, all parents who gave consent to dust sampling, with a maximum number of 1000 per country, were sent nylon sample socks to collect dust with, according to detailed photo-instructions. In this way, house dust of 3,625 families was collected but only dust socks from circa 500 children with wheeze and 500 without wheeze were analyzed. Figure 2 gives a schematic overview of the PARSIFAL population, including the sub-populations in which dust was collected, and indicates for each chapter of this thesis, which sub-population was used.

The aim of the research described in this thesis was to investigate whether, within the PARSIFAL study, differences in atopic disease prevalences between farm children or children from anthroposophic families and respective references could be explained by differences in microbial agent levels and/or pet and mite allergen levels in house dust.

OUTLINE THESIS

Chapter 2 describes the differences in allergic diseases and atopic sensitization between farm children, children from anthroposophic families (‘Steiner children’) and respective references of the PARSIFAL population. Included allergic disease outcomes, as assessed by questionnaire, were: current rhinoconjunctivitis (‘hay fever’) symptoms, doctor’s diagnosis of current rhinoconjunctivitis, current wheezing, doctor’s diagnosis of asthma, current atopic eczema symptoms and doctor’s diagnosis of current atopic eczema. Atopic sensitization was assessed by IgE serology.

Chapter 3 evaluates whether there are differences in bacterial and fungal agent levels, i.e. endotoxin, ß(1,3)-glucans and EPS, in house dust between farm, Steiner and reference children. Also differences between the 5 countries were evaluated. Furthermore, it was investigated whether exposure differences between the study groups and between countries could be explained by parent-reported differences in home and family characteristics.

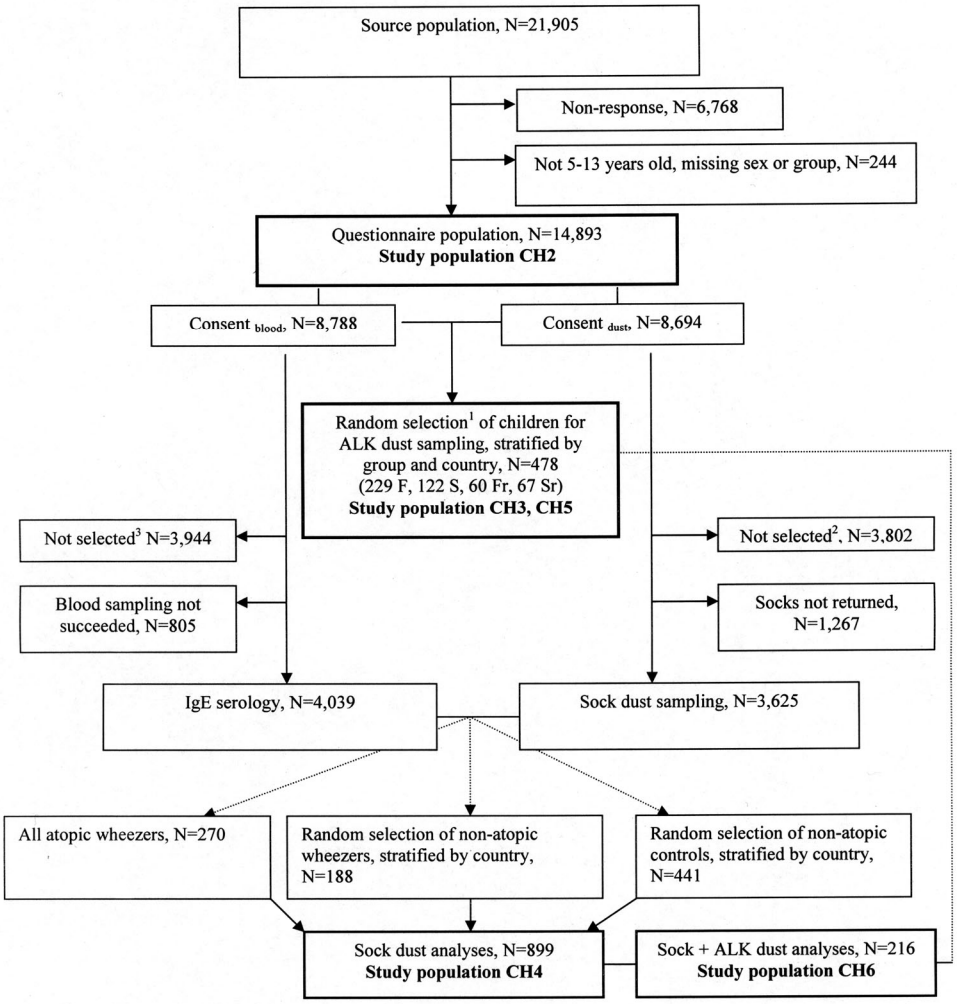

Figure 2. Schematic overview of the PARSIFAL population, indicating which subpopulations were included in each chapter of this thesis (CH=Chapter, F=Farm, S=Steiner, Fr=Farm reference, Sr=Steiner reference children).

1. *Farms without livestock were excluded. AU: only consent to dust sampling required. NL+SE: restrictions to some geographical areas.*
2. *CH and GE made selections, because of their relatively large populations. CH: 1000 children, incl. all with consent to dust and blood sampling; GE: 350 F (consent to dust+blood), 368 S, 150 Fr, 170 Sr.*
3. *Selection of: CH 865 children + 38 extra wheezers; GE: all F, 368 S (incl all wheezers), 150 Fr, 150 Sr (incl all wheezers).*
   (SE=Sweden, CH=Switzerland, NL=the Netherlands, GE=Germany, AU=Austria)

Chapter 4 describes the association between house dust levels of bacterial and fungal agents, i.e. endotoxin, ß(1,3)-glucans and EPS, and atopic wheeze in children of the PARSIFAL population. It also describes to what extent differences in wheezing prevalences between the subgroups (farm, Steiner and reference children) could be explained by microbial agent levels.

Chapter 5 evaluates whether there is a dose-response relation between mite allergen levels in house dust and mite specific sensitization and whether this relation is different in the various subgroups of the PARSIFAL population (farm, Steiner and reference children). It also evaluates whether bacterial and fungal agents in house dust modified this relationship and thereby might protect against sensitization.

Chapter 6 compares the two ways in which dust was collected in the PARSIFAL study; with ALK devices by fieldworkers, of which results have been described in Chapter 3 and Chapter 5, and with nylon socks by parents, of which results have been described in Chapter 4. For the 216 homes in which both methods were applied, house dust levels of pet and mite allergens and bacterial and fungal agents were compared between the methods. Additionally, it was investigated whether differences between the methods could be attributed to the use of different sampling devices by including analysis of extra sock samples, taken by fieldworkers on the same day as the ALK samples.

Chapter 7 discusses the results of this thesis and brings us back to the question what is still to be discovered with regard to atopic diseases, in the infinity of the infinitesimal: dust.

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CHAPTER 2

Allergic diseases and atopic sensitization in children related to farming and anthroposophic lifestyle – the PARSIFAL study

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ABSTRACT

Background The prevalence of allergic diseases has increased rapidly in recent decades, particularly in children. For adequate prevention it is important not only to identify risk factors, but also possible protective factors. The aim of this study was to compare the prevalence of allergic diseases and sensitization between farm children, children in anthroposophic families, and reference children, with the aim to identify factors that may protect against allergic disease.

Methods The study was of cross-sectional design and included 14,893 children, aged 5-13 years, from farm families, anthroposophic families (recruited from Steiner schools) and reference children in Austria, Germany, the Netherlands, Sweden and Switzerland. A detailed questionnaire was completed and allergen-specific IgE was measured in blood.

Results Growing up on a farm was found to have a protective effect against all outcomes studied, both self-reported, such as rhinoconjunctivitis, wheezing, atopic eczema and asthma, and sensitization (allergen specific IgE $\geq$ 0.35 kU/L). The adjusted odds ratio for current rhinoconjunctivitis symptoms was 0.50 (95% CI 0.38-0.65) and for atopic sensitization 0.53 (95% CI 0.42-0.67) for the farm children compared to their references. The prevalence of allergic symptoms and sensitization was also lower among Steiner school children compared to reference children, but the difference was less pronounced and not as consistent between countries, adjusted OR for current rhinoconjunctivitis symptoms was 0.69 (95% CI 0.56 -0.86) and for atopic sensitization 0.73 (95% CI 0.58-0.92).

Conclusions This study indicates that growing up on a farm, and to a lesser extent leading an anthroposophic life style, may confer protection from both sensitization and allergic diseases in childhood.

INTRODUCTION

The prevalence of allergic asthma, allergic rhinoconjunctivitis, and atopic eczema has increased markedly in recent decades, particularly among children [1-2], although there is some recent evidence that the prevalence rates are stabilising [3-5]. The total costs for the major allergic diseases are estimated to 10 billion Euros for direct costs and 19 billion Euros for indirect costs in Europe [6]. For adequate prevention it is important not only to identify risk factors, but also possible protective factors.

The PARSIFAL (Prevention of Allergy - Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) project focuses on two groups of children who have shown a lower prevalence of atopic diseases and sensitization: farm children [7-11] and children in families with an anthroposophic lifestyle [12]. Contact with farm animals, especially in early childhood, has been associated with a decrease in the risk of

atopic disease [8, 9, 13, 14]. This protective effect might partly arise through exposure to microbial compounds [15-18].

The anthroposophic way of life involves several characteristics that were more common in the general population some decades ago, such as restrictive use of antibiotics, antipyretics and vaccinations, as well as certain dietary habits. In a previous Swedish study it was not possible to identify any single lifestyle factor as primarily responsible for the lower prevalence of atopy, because the behavioural characteristics of the anthroposophic lifestyle were strongly correlated [12].

In this article we compared the prevalence of allergic diseases and sensitization in children from farm or anthroposophic families and their respective reference groups in five European countries (Austria, Germany, the Netherlands, Sweden and Switzerland). The study is larger than most previous studies; moreover farming practices and the lifestyle of the anthroposophic communities differ between the countries, enhancing the resolution power of identifying important determinants of the lower disease rates.

METHODS

Study design

Children, aged 5-13 years, from farm families or attending Steiner schools were compared with children from appropriate reference groups in Austria, Germany, the Netherlands, Sweden and Switzerland. Children in Steiner schools often come from families with an anthroposophic lifestyle. Farm children were defined as children currently living on a farm and whose family run the farm. In Austria, children of farmers and reference children not designated as farm children were selected from schools in rural areas by their teachers, who had a good knowledge of the pupils and their parents. In Germany, the Netherlands and Switzerland children were selected from schools in rural areas known to have a high percentage of farmers. All farm children in the selected schools were included in these countries and all of the farm reference children were included in Germany and the Netherlands, whereas in Switzerland only a random sample of the farm reference children were included. In Sweden, farmers with children, in selected areas, were identified from the Farming Registry at the National Bureau of Statistics and farm reference children were randomly selected from the population registry among children living in the same area. In all countries, children with an anthroposophic lifestyle were recruited from classes in Steiner schools. All children in Steiner classes with children of the right age were invited in the selected schools. Corresponding reference children were selected from other schools in the vicinity. After a first sampling seven Steiner schools and one reference school, all located in Germany, were excluded because of low participation rate, which appeared to be due to lack of interest on the part of the school rather than refusal by individuals.

Questionnaire and clinical examination

The parents completed a detailed questionnaire, which included questions on environmental exposures, lifestyle, socio-economic conditions, history of infections, diet, contact with animals, and on symptoms of bronchial asthma, rhinoconjunctivitis and atopic eczema. Most of the questions were based on the internationally validated and translated ISAAC phase II questions [19] and the Swedish BAMSE study [20]. Questions on exposures and lifestyle factors related to living on a farm were largely from the earlier ALEX study in Switzerland, Germany and Austria [13] and questions regarding factors associated with anthroposophic lifestyle were based on a Swedish study [12]. The questionnaires were distributed and collected from October 2000 to May 2002, during largely overlapping time periods in the five countries.

In Austria, the Netherlands and Sweden all children whose parents had consented to blood sampling were invited to a clinical examination. In Germany and Switzerland only a random sample of those who consented were invited, because of the comparatively large number of children included in these countries. In Germany there was also a selection among the Steiner children, only children whose parents had reported an anthroposophic lifestyle were included. The clinical examination was performed by specially trained nurses and included blood samples, measurement of weight and height as well as collection of information about the child's immunizations.

Allergen-specific IgE was measured against a mix of common inhalant allergens (Phadiatop; allergens included are *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, birch, timothy, mugwort, cat, dog, horse, *Cladosporium herbarum*) and a mix of common food allergens (hen's egg white, codfish, cow's milk, peanut, soy bean, wheat flour) (Pharmacia CAP System, Pharmacia Diagnostics AB, Uppsala, Sweden). All analyses were performed at the Department of Clinical Immunology, Karolinska University Hospital, Stockholm, Sweden.

Endpoint definitions and statistical analyses

All health endpoints were self-reported by the parents of the children except atopic sensitization which was assessed by blood samples. Children were considered to have current rhinoconjunctivitis symptoms if sneezing, runny nose, stuffy nose and itchy eyes were reported in the last 12 months, without the child having a cold at the same time. Current wheezing was defined as at least one episode of wheezing during the last 12 months. Current atopic eczema symptoms were considered present if the child had ever had an itchy rash intermittently for at least 6 months at any time during the last 12 months. Children reported to ever have had symptoms of seasonal rhinoconjunctivitis and been diagnosed with seasonal rhinoconjunctivitis, were considered to have a doctor's diagnosis of rhinoconjunctivitis. Children with an intermittent itchy rash lasting at least 6 months and who had been diagnosed with atopic/allergic eczema were considered to have a doctor's

diagnosis of atopic eczema and children who had ever been diagnosed with asthma, or with obstructive bronchitis more than once, were considered to have a doctor's diagnosis of asthma. IgE values $\geq$ 0.35 kU/L in either Phadiatop and/or the mix of common food allergens defined atopic sensitization.

Statistical analyses were performed using Stata (version 8.0, Stata Corp LP, College Station, TX, USA) and SAS (version 8.1, SAS Institute, Inc., SAS Software). Statistical significance was defined as a two-sided p-value of < 0.05, using Chi square analyses. Odds ratios with 95% confidence intervals were computed using logistic regression analysis. In the logistic regression analyses adjustments were made for country, sex, age (5 categories), mother's reported asthma and/or rhinoconjunctivitis, father's reported asthma and/or rhinoconjunctivitis, parental education (3 categories), maternal smoking during pregnancy, current smoking in the household and older siblings (4 categories).

The study was approved by local research ethics committees in each country and informed consent was obtained from the parents of each child.

RESULTS

Of the 21,905 children invited to take part in the study 15,137 (69%) provided questionnaire data (table 1).

Table 1. Participation rates in the PARSIFAL study, subdivided by country.

Country	No. of selected children	No. of obtained questionnaires	Participation rate questionnaires (%)	Consent for blood sample	Blood samples	Participation rate blood samples (%)
Austria	1,589	1,282	80.7	806	759	47.8
Germany	9,240	6,963	75.4	5,045[a]	1,159	40.9[b]
The Netherlands	6,403	3,230	50.4	691	552	8.6
Sweden	1,550	1,109	71.5	944	836	53.9
Switzerland	3,123	2,553	81.7	1,302[a]	743	35.8[b]
Total	**21,905**	**15,137**	**69.1**	**8,788**	**4,049**[c]	**33.5**

a. In Germany and Switzerland only a random sample of the children who had consented to give a blood sample was selected (1548 and 865 respectively).
b. Calculated as (no. of blood samples/selected for blood samples) x (consent for blood samples/no. of selected children).
c. 10 children missing group information.

The combined participation rate for farm children and farm reference children was 70%. The corresponding figure for Steiner children was 67% and for Steiner reference children 70%. The participation rate ranged from 50% in the Netherlands to 82% in Switzerland (table 1), and did not differ to any major extent between the subgroups within each country (data not shown). A total of 237 children were excluded because they were outside the age limits (5-13 years old) and seven children because of missing information regarding sex or which group they belonged to, leaving 14,893 children for the analyses.

Table 2. Characteristics of children in the PARSIFAL study subdivided by study group.

	Farm children	Farm reference children	Steiner children	Steiner reference children	All children
Number of children (N)	2,823	5,440	4,606	2,024	14,893
Sex (% males)[a]	51.5	51.4	48.0	49.2	50.0
Age (years)[a]	9.0	8.7	9.1	8.8	8.9
Older siblings (number)[a]	1.3	0.8	1.1	0.8	1.0
Height (cm)[a]	139.3	137.1	139.2	137.7	138.3
Weight (kg)[a]	34.2	32.0	32.2	32.5	32.5
Mother's reported asthma or rhinoconjunctivitis[a,b] (% yes)	12.4	18.6	26.9	24.0	20.7
Father's reported asthma or rhinoconjunctivitis[a,b] (% yes)	9.3	15.8	26.0	23.8	18.8
Parental education[a,c]					
Gymnasium (%)	53.7	51.9	31.2	47.4	45.2
University (%)	11.1	18.3	66.4	39.2	34.7
Maternal smoking during pregnancy (%)[a]	6.4	12.4	8.1	13.0	10.2
Current smoking in the household (%)[a,d]	15.6	24.6	13.8	19.3	19.9

a. Computed among those answering a specific question. Internal non-response/missing rate was approximately 11% for height and weight and less than 3% for all other characteristics.
b. Defined as having or having had asthma and/or rhinoconjunctivitis.
c. Three groups according to the highest level of education of the mother and father: elementary school or lower, gymnasium/secondary school and university education.
d. Mother, father or someone else smokes regularly in the child's home.

In all 8,788 children gave consent for blood sampling and of these 4,854 were invited for blood sampling and 4,049 gave a blood sample. A total of 10 were excluded because information regarding group was missing. Overall there were no consistent differences in the prevalence of various allergic symptoms between those who provided blood samples and those who did not (data not shown). However, while the prevalence of any allergic symptoms or doctor's diagnosed disease was similar among those with and without blood samples from the Steiner children (30% and 29% respectively), it appeared higher for those with blood samples (36%) than those without (31%) in the Steiner reference group. These differences in symptoms/disease rates related to blood samples between Steiner and Steiner reference children tended to be most pronounced in Sweden, Switzerland and the Netherlands.

There were clear differences between the four groups of children in terms of heredity (table 2). Both maternal and paternal reported asthma or rhinoconjunctivitis was significantly more common in the Steiner and Steiner reference group than in the farm reference group and was most rare in the farm group. University education was most common among the parents of the Steiner children. Both maternal smoking and current smoking in the household were more common in the reference groups than in the farm and Steiner groups. The country-specific results regarding differences between the four groups in relation to the characteristics described in table 2 were largely consistent (not shown in table).

The prevalence of various symptoms of allergy and doctor's diagnosis of allergic disease as well as atopic sensitization is illustrated in table 3. The prevalence for all outcomes was significantly lower in the farm group than in the reference group, except for current atopic eczema symptoms where the difference was of borderline significance. Similarly, the Steiner children showed significantly lower prevalences of all outcomes, except current wheezing and doctor's diagnosis of atopic eczema, as compared to their reference children.

Substantial differences in prevalence of allergic diseases and sensitization were evident between countries and exposure groups (fig. 1 a-g). However, in all countries the lowest prevalence rates tended to be found among the farm children, with the most consistent differences between the farm children and the farm reference children for rhinoconjunctivitis and atopic sensitization (fig. 1 a,b,g). The effects of group belonging on doctor's diagnosis of asthma prevalence varied with the most pronounced differences in Germany (fig. 1 d). The effects of the differences between the Steiner children and the Steiner reference children also varied from country to country. The most consistent differences in prevalence between the Steiner children and their references across several outcomes were seen in Germany, the Netherlands and Sweden.

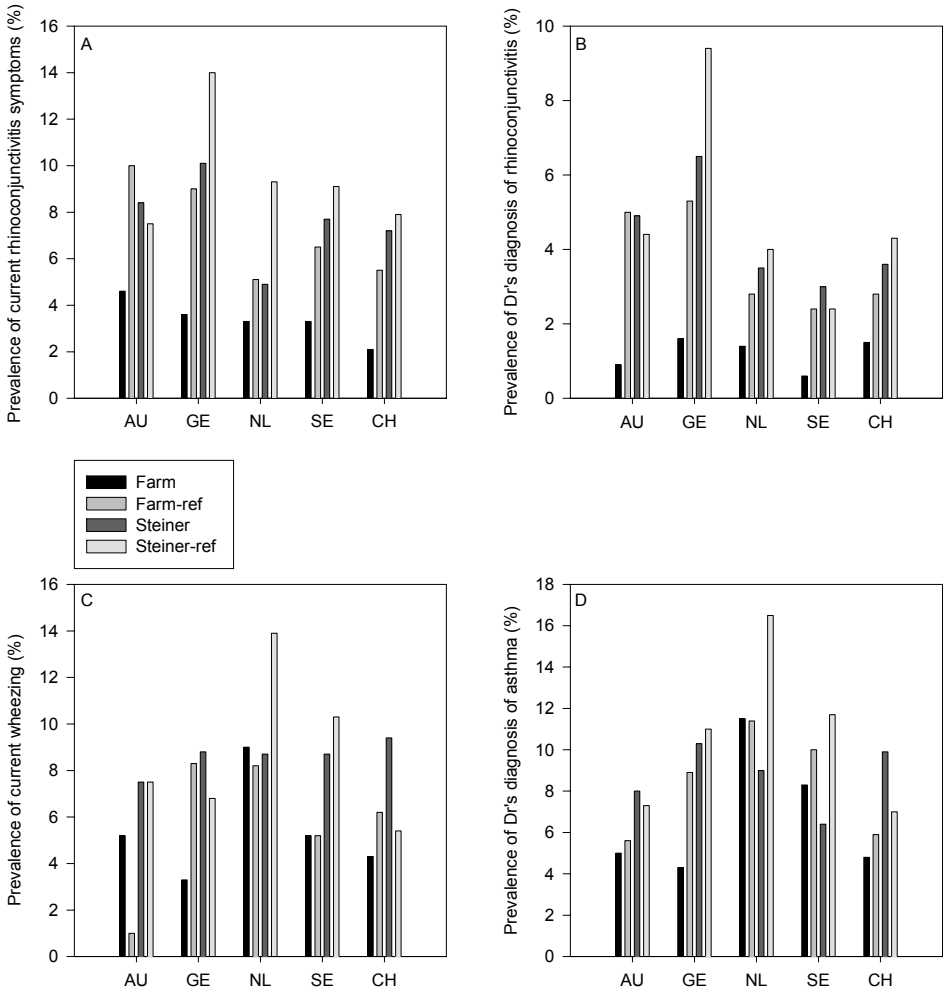

Figure 1 a-d. a) Prevalence of current rhinoconjunctivitis symptoms, b) Doctor's diagnosis of current rhinoconjunctivitis, c) Current wheezing, d) Doctor's diagnosis of asthma, e) Current atopic eczema symptoms, f) Doctor's diagnosis of current atopic eczema, and g) Atopic sensitization (IgE ≥0.35 kU/L in Phadiatop and/or mix of common food allergens), subdivided by country and study group (GE=Germany, SE=Sweden, AU=Austria, NL=The Netherlands, CH=Switzerland).

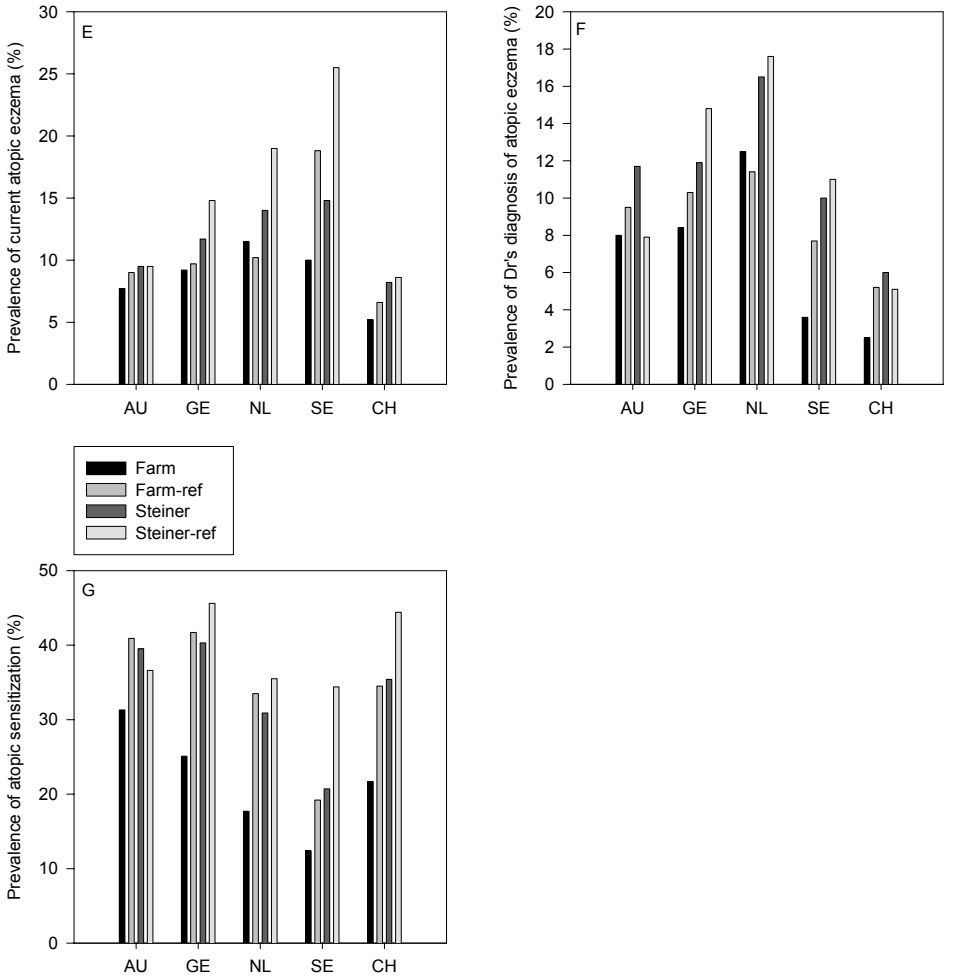

Figure 1 e-g. a) Prevalence of current rhinoconjunctivitis symptoms, b) Doctor's diagnosis of current rhinoconjunctivitis, c) Current wheezing, d) Doctor's diagnosis of asthma, e) Current atopic eczema symptoms, f) Doctor's diagnosis of current atopic eczema, and g) Atopic sensitization (IgE $\geq$0.35 kU/L in Phadiatop and/or mix of common food allergens), subdivided by country and study group (GE=Germany, SE=Sweden, AU=Austria, NL=The Netherlands, CH=Switzerland).

Table 3. Prevalence of allergic diseases and atopic sensitization[a], for all PARSIFAL children subdivided by study group.

	Farm children		Farm reference children		Steiner children		Steiner reference children	
	%	n/N[b]	%	n/N[b]	%	n/N[b]	%	n/N[b]
Current rhinoconjunctivitis symptoms	3.3	91/2,801	7.7	416/5,373	8.0	363/4,558	10.6	212/1,998
Doctor's diagnosis of rhinoconjunctivitis	1.3	37/2,789	4.4	236/5,347	4.8	216/4,532	6.1	121/1,987
Current wheezing	5.0	140/2,795	7.7	412/5,374	8.8	398/4,548	8.4	168/2,002
Doctor's diagnosis of asthma	6.3	172/2,750	9.1	484/5,330	9.3	421/4,551	11.0	217/1,979
Current atopic eczema symptoms	8.6	239/2,791	9.7	526/5,399	11.6	525/4,540	14.6	294/2,011
Doctor's diagnosis of atopic eczema	7.1	198/2,790	9.9	535/4,848	11.5	521/4,004	12.3	246/2,001
Atopic sensitization[a]	22.7	314/1,386	34.7	243/701	32.2	387/1,201	39.1	248/634

a. IgE≥0.35kU/L in Phadiatop and/or mix of common food allergens.
b. n=number of children with actual outcome, N=total number of responses to the question/in the analyses.

The odds ratios for the studied health outcomes, comparing the farm group and the Steiner group with their respective reference groups, are shown in table 4. For the farm group, the adjusted odds ratios were significantly lower than in the reference group for all the studied health outcomes, except current atopic eczema symptoms and doctor's diagnosis of eczema, where the results were of borderline significance. For instance, the adjusted OR for current rhinoconjunctivitis symptoms was 0.50 (95% CI 0.38-0.65) and for atopic sensitization 0.53 (95% CI 0.42-0.67). Among the Steiner children the adjusted odds ratios were significantly lower for current rhinoconjunctivitis symptoms, doctor's diagnosis of rhinoconjunctivitis, current atopic eczema symptoms and atopic sensitization, and of borderline significance for doctor's diagnosis of asthma and doctor's diagnosis of atopic eczema. In this group the adjusted OR for current rhinoconjunctivitis symptoms was 0.69 (95% CI 0.56-0.86) and for atopic sensitization 0.73 (95% CI 0.58-0.92). When adjustment was made for prevalence of allergic symptoms or doctor's diagnosed disease, to minimize potential selection bias in blood sampling, the OR for atopic sensitization was 0.79 (95% CI

0.62-1.01). Additional adjustment for height and weight did not result in any notable changes in the odds ratios.

Table 4. Odds ratios (OR) and confidence intervals (CI) for allergic diseases and sensitization among farm children and Steiner children compared to their respective reference groups.

	Unadjusted OR[a]				Adjusted OR[b]			
	Farm vs Farm-ref		Steiner vs Steiner-ref		Farm vs Farm-ref		Steiner vs Steiner-ref	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Current rhinoconjunctivitis symptoms	0.43	0.33-0.54	0.73	0.61-0.87	0.50	0.38-0.65	0.69	0.56-0.86
Doctor's diagnosis of rhinoconjunctivitis	0.33	0.23-0.48	0.78	0.62-0.98	0.39	0.26-0.58	0.72	0.55-0.94
Current wheezing	0.70	0.57-0.86	1.03	0.86-1.26	0.78	0.62-0.99	1.10	0.89-1.36
Doctor's diagnosis of asthma	0.70	0.58-0.85	0.83	0.70-0.99	0.74	0.60-0.92	0.85	0.69-1.03
Current atopic eczema symptoms	0.90	0.76-1.07	0.75	0.64-0.87	0.89	0.74-1.07	0.68	0.57-0.81
Doctor's diagnosis of eczema	0.82	0.69-0.98	0.92	0.78-1.08	0.83	0.68-1.01	0.88	0.73-1.06
Atopic sensitization	0.53	0.42-0.65	0.77	0.63-0.95	0.53	0.42-0.67	0.73	0.58-0.92

a. Only adjusted for country.
b. Adjusted for country, sex, age, mother's reported asthma and/or rhinoconjunctivitis, father's reported asthma and/or rhinoconjunctivitis, parental education, maternal smoking during pregnancy, current smoking in the household, older siblings.

DISCUSSION

This large cross-sectional study among children aged 5-13 years in Austria, Germany, the Netherlands, Sweden and Switzerland indicated that growing up on a farm, and to a lesser extent leading an anthroposophic lifestyle, is protective against both atopic sensitization and childhood allergic diseases.

The beneficial influence of growing up on a farm against sensitization and development of allergic diseases in childhood has earlier been reported in studies from Austria, Canada, Finland, Germany, Sweden and Switzerland [7-10, 13, 21, 22]. The farming

environment appeared to provide more consistent protection against rhinoconjunctivitis and sensitization than against asthma and other atopic diseases [7-10, 13, 21, 22], and this was confirmed by our study. Farm children are more exposed to micro-organisms related to livestock animals, which has been proposed to protect against developing sensitization and allergic diseases [13, 15, 16]. In addition, long-term and early life exposure to stables and farm milk has been shown to protect against the development of asthma, rhinoconjunctivitis and atopic sensitization [13].

We also reported a lower prevalence of all studied outcomes, with the exception of current wheezing, among children attending Steiner schools (as a proxy for leading an anthroposophic lifestyle). However, these results tended to vary more between the countries, possibly because the anthroposophic lifestyle to some extent differs between the countries and that the families who have their children in Steiner schools are more or less anthroposophic. Nevertheless, our results confirm previous findings from Sweden [12]. The lifestyle of children in anthroposophic families differs with respect to several characteristics that may be of importance for allergy, e.g. restrictive use of antibiotics, antipyretics and certain vaccinations, as well as dietary habits including intake of fermented vegetables and biodynamic food. The prevalence of allergic diseases, but not atopic sensitization, was slightly lower in the farm reference group than in the Steiner group. This could possibly be explained by the fact that most of the Steiner schools were in urban or sub-urban areas and the farm reference group lived in a rural setting, as earlier studies have shown a lower prevalence of allergic diseases in rural than in urban areas [23]. However, the study was not designed to test this hypothesis, so no further comparisons between these groups have been made.

It is important to consider the possibilities of bias. The questions regarding symptoms and diagnosis of asthma, rhinoconjunctivitis and atopic eczema were based on the internationally validated and translated ISAAC phase II questions [19]. We used the overall denotion ‘allergic diseases’ to characterize these conditions, primarily based on questionnaire responses regarding symptoms and/or doctor's diagnosis [24]. The response rate for the questionnaire was high in all countries except the Netherlands, where it was 50%. The participation rate in blood sampling was also low there, which may partly be related to outbreaks of Foot and Mouth Disease during the recruitment phase, as well as recently changed Institutional Review Board requirements. However, our data on the prevalence of the studied outcomes in the Netherlands were comparable with the results from the Dutch part of the ISAAC-II study [25] where the participation rate was higher (65%), speaking against important selection bias. In the assessment of results based on the blood samples among the Steiner and Steiner reference groups, e.g. atopic sensitization, there is additional concern for possible selection bias since participation seemed to be related to allergic symptoms or doctor’s diagnosed disease. However, adjustment for symptoms or disease prevalence, to minimize disease related selection bias, resulted in a small effect on odds

ratios. Considering also that this represents an overadjustment, it speaks against a major effect by selection bias. The 'healthy farmer effect', i.e. a selective avoidance of farming by atopic families, may be a problem. The reported prevalence of asthma and/or rhinoconjunctivitis was lower among parents in the farm group than in the other studied groups. This might in part be explained by families with atopic diseases leaving their farms, although we do not have supporting evidence. Another possibility is that farming protects against atopy not only among children but also among adults, or that the childhood protection once similarly afforded to the parent generation extends into their adulthood.

An ultimate aim of epidemiological research on allergic diseases is to better understand and identify which factors in the environment and lifestyle are responsible for the increase in prevalence of these diseases over the recent decades. There is most probably no single factor in the farm and/or anthroposophic environment that prevents children from developing allergic diseases, but rather that the effects result from a complex interplay between various environmental and lifestyle factors. The rich database and the differences between the countries in the present study will permit further studies into specific possible protective factors.

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CHAPTER 3

Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children – the PARSIFAL study.

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ABSTRACT

Background Growing up on a farm and an anthroposophic lifestyle are associated with a lower prevalence of allergic diseases in childhood. It has been suggested that the enhanced exposure to endotoxin is an important protective factor of farm environments. Little is known about exposure to other microbial components on farms and exposure in anthroposophic families.
Objective To assess the levels and determinants of bacterial endotoxin, mould ß(1,3)-glucans and fungal extracellular polysaccharides (EPS) in house dust of farm children, Steiner school children and reference children.
Methods Mattress and living room dust was collected in the homes of 229 farm children, 122 Steiner children and 60 and 67 of their respective reference children in 5 European countries. Stable dust was collected as well. All samples were analyzed in one central laboratory. Determinants were assessed by questionnaire.
Results Levels of endotoxin, EPS and glucans per gram of house dust in farm homes were 1.2 – 3.2 fold higher than levels in reference homes. For Steiner children, 1.1 – 1.6 fold higher levels were observed compared to their reference children. These differences were consistently found across countries, although mean levels varied considerably. Differences between groups and between countries were also significant after adjustment for home and family characteristics.
Conclusion Farm children are not only consistently exposed to higher levels of endotoxin, but also to higher levels of mould components. Steiner school children may also be exposed to higher levels of microbial agents, but differences with reference children are much less pronounced than for farm children. Further analyses are however required to assess the association between exposure to these various microbial agents and allergic and airway diseases in the PARSIFAL population.

INTRODUCTION

Several studies have shown that growing up on a farm protects against the development of atopic diseases [1-6]. Contact to livestock has been suggested to account for this association [1, 2]. It has been speculated that farm children are exposed to higher levels of microbial compounds, which may stimulate innate immunity and suppress atopic sensitization. Indeed, elevated levels of endotoxin, an intrinsic part of the outer membrane of gram-negative bacteria, have been found in mattresses of farm children as compared to non-farm children in Germany, Austria and Switzerland. Also non-farm children with regular contact to farm animals were exposed to elevated endotoxin levels [7].

The same study showed that the endotoxin levels in mattress dust were inversely related to the prevalence of hay fever, atopic asthma, and atopic sensitization [8]. An increased expression of receptors for microbial compounds (Toll-like receptor 2 and CD14) in farm children was also observed, and thought to be due to the higher endotoxin exposure [9]. However, the Toll-like receptors are not specific for endotoxin [9], and the observed changes may thus also be due to other microbial components, like ß(1,3)-glucans, cell wall constituents of most fungi, with known immunomodulatory effects [10-15].

Occupational studies have shown that farmers are exposed to high levels of both bacteria and fungi in animal houses [16, 17] but little is known of their domestic exposure. We aimed to investigate whether the levels of fungal components in house dust are, like endotoxin, higher in farm children as compared to reference children. We therefore assessed levels of house dust-associated ß(1,3)-glucans and extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp.*, which both have been shown to be associated with total culturable fungi in house dust, and thus may be good markers for indoor fungal exposure [18]. We further studied whether differences in bacterial and fungal exposure between farm children and reference children would be similar in 5 European countries, with large differences in farming practices, which may influence indoor microbial agent levels differently [19, 20].

A low prevalence of atopic diseases and sensitization has also been found in children attending Rudolf Steiner schools, who predominantly come from families adhering to an anthroposophic lifestyle [21]. While that study showed an inverse relation between the number of characteristic features of an anthroposophic lifestyle (like restricted use of antibiotics and immunizations) and risk of atopy [21], no attention was given thus far to possible differences in exposure to house dust-associated microbial agents in this population. We therefore also assessed levels of these agents in house dust from Rudolf Steiner school children and reference children.

Finally, we investigated whether exposure differences between the study groups and between countries could be explained by parent-reported differences in home and family characteristics.

METHODS

PARSIFAL

In 2000, the cross-sectional PARSIFAL (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) study on the association between environmental and lifestyle factors and allergies and asthma was initiated. The goal of PARSIFAL is to explore factors characteristic of farming and anthroposophic populations that may protect against the development of atopic diseases in

children. A total of 14,893 children aged 5-13 years were included among farmers, families with an anthroposophic lifestyle, and respective reference groups in five European countries: Austria, Germany, the Netherlands, Sweden, and Switzerland. Children with an anthroposophic lifestyle were recruited through Rudolf Steiner schools. The reference children were selected such that they lived in the neighborhood of farm and Steiner school children, but did not actually live on a farm or did not attend a Rudolf Steiner school, respectively. Exposure information like home and farm characteristics was assessed through a questionnaire administered to the parents of the participating child. The study design further included collection of house dust in a sub-sample of 100 children per country: 50 farm children, 25 children of Rudolf Steiner schools and 12 to 13 children of each reference group. Farms without livestock (representing less than 20% of the farm children questionnaire population) were excluded from the selection of families for dust sampling. The Steiner and reference children were randomly selected from the children whose parents consented to dust sampling and blood sampling (more than 50% of the questionnaire population). Selection procedures differed slightly between countries; in Austria consent to blood sampling was not required and in Sweden and the Netherlands the selection was restricted to some geographical areas. The subpopulation, selected for dust sampling, had essentially the same distribution of determinants (like age of the home, contact to farm animals etcetera) as the total PARSIFAL population. In total, fieldworkers collected dust from the mattresses and living room floors of 229 farm children, 122 Steiner children and 60 and 67 of their respective reference children. On farms, dust from the animal stable(s) was collected as well.

Dust collection

Dust from mattresses and living room floors was collected on pre-weighed glass fibre filters using vacuum cleaners with sampling nozzles (ALK, Horsholm, Denmark) according to a standardized protocol with photo- and video-instructions. The whole area of the mattress (with under-sheets only) was vacuumed for 2 minutes. For living room floors, sampling time and area depended on type of floor covering; carpeted floors, 1 m², 2 min; smooth floor with ≥ 4 m² rug, 1 m² of rug, 2 min; smooth floor with no rug or smaller rug(s), 2 m² of smooth floor, 4 min. Stable dust was collected at 0.5-1.5 m above the floor from various surfaces (shelves, window sills etc.), using a brush and a dust pan. After each sampling of mattress-, living room- or stable dust all sampling tools (ALK nozzle or brush and dust pan) were cleaned thoroughly with 70% ethanol. All samples (stable dust or filters with sampled house dust) were stored in tightly closed new, disposable 50 ml tubes, stored frozen at the various centers, and shipped on dry ice to one laboratory (IRAS, Utrecht, NL), where samples were stored at –20 ^{0}C for 4 up to 19 months until extraction. To assess the risk of contamination during sampling and sample processing procedures, each centre included during the fieldwork a number of 'field blanks' (total n=41): pre-weighed filters in 50 ml

tubes that were transported to field locations, opened and further treated as sample filters, except that no actual sampling was performed.

Dust extraction

For house dust samples, filters plus dust were weighed and then extracted in a volume of 5 to 40 ml, determined by the net dust weight (<0.5 g, 5 ml; 0.5 to 1.0 g, 10 ml; 1.0 to 2.0 g, 20 ml; >2.0 g, 40 ml). Stable dust was sieved through a 0.425 mm mesh and 150 mg of each sample was extracted in a volume of 5 ml. Endotoxin, EPS and glucans were extracted sequentially, essentially as described previously [22-24]. First, 5 to 40 ml 0.05% (v/v) Tween-20 in pyrogen-free water was added, suspensions were incubated in an end-over-end roller for 1 h at room temperature, and after centrifugation (15 min, 1,000 x g) the upper 10% of supernatant was harvested and stored in 4 aliquots at –20 °C for endotoxin analysis. For the second step, the removed supernatant was replaced with the same volume of 10*concentrated phosphate-buffered saline (PBS), thus changing the extraction medium into PBS-0.045% Tween-20. After re-suspension and thorough mixing of the first pellet in the new medium, incubation was continued in the end-over-end roller (1h), followed by centrifugation (15 min, 2,000 x g). The supernatant was harvested and stored in 6-10 aliquots at –20 °C for analysis of EPS and pet and mite allergens. The remaining dust pellets were stored at –20 °C. For the extraction of glucans, the pellets were re-suspended in the original volume of PBS-Tween (0.05%), incubated in an end-over-end roller for 15 min, autoclaved for 1 h at 120 ^{o}C and incubated in an end-over-end roller again for 15 min. After centrifugation (15 min, 1,000 x g), supernatant was harvested and stored in 2 aliquots at –20 °C until analysis.

Analysis of microbial components

Endotoxin was analyzed with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, using the same batch of LAL reagents and standards for all analyses (BioWhittaker, LAL lysate lot no. 1L676S, LPS standard lot no. 2L0090) [22]. EPS was analyzed with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium spp.* [23] and glucans were measured with an inhibition EIA [24]. Concentrations were expressed as endotoxin units [22], EPS units (based on an in-home standard consisting of a pooled house dust extract given an arbitrary value of 5,000 EPS units/ml [22]) and micrograms of glucans per gram of dust and per square meter. For stable samples, levels of all three components were only calculated per gram of dust. The average inter-day/inter-assay coefficients of variation, as determined by testing duplicate extract aliquots of 10% of all samples on another day as the first aliquot, ranged from 14.5-30.5%. The 41 field blanks (which were treated exactly the same as the other filters (including transport to homes etc.), except dust sampling) showed non-detectable (90-95%) or very low levels of microbial agents.

Amounts of dust lower than 0.020 g were considered undetectable and were given a value of 0.013 g. All mattress samples had a detectable amount of dust. For living room (n=25) and stable (n=15) samples with undetectable amounts of dust, no concentrations per gram of dust were calculated, unless the amounts of microbial components were undetectable too. Samples with non-detectable amounts of endotoxin, EPS or glucans (n=18, n=14 and n=9 respectively) were given a value of two-thirds of the lowest observed detectable amount per gram of dust or per square meter for the specific component determined. Overall, less than 5% of results were missing, because of the undetectable amounts of dust or sampling or extraction failures, or because the surface area of the mattress had not been recorded.

Statistical analysis

Levels of microbial components were approximately normally distributed after natural log (ln)-transformation. The differences between groups were evaluated by performing Student's t-tests, with ln-transformed values. Correlations were evaluated by calculating Pearson correlation coefficients with ln-transformed values. The associations between the ln-transformed levels and self-reported home, farm and family characteristics were studied in univariate and multivariate regression analyses. The association of each dichotomous variable (set at 1 for children with that characteristic and 0 for children without that characteristic) with the level of any of the microbial agents was expressed as the ratio of covariate-adjusted geometric mean levels of that microbial agent for children with and children without that characteristic (means ratio). Characteristics that did not have a significant effect on any of the microbial component levels were left out of the final multivariate models because of power implications. To test for heterogeneity in the observed associations, the multivariate regression models were also run for each country and study group separately. SAS statistical software (version 8.2, SAS Institute, Cary, N.C.) was used for all analyses.

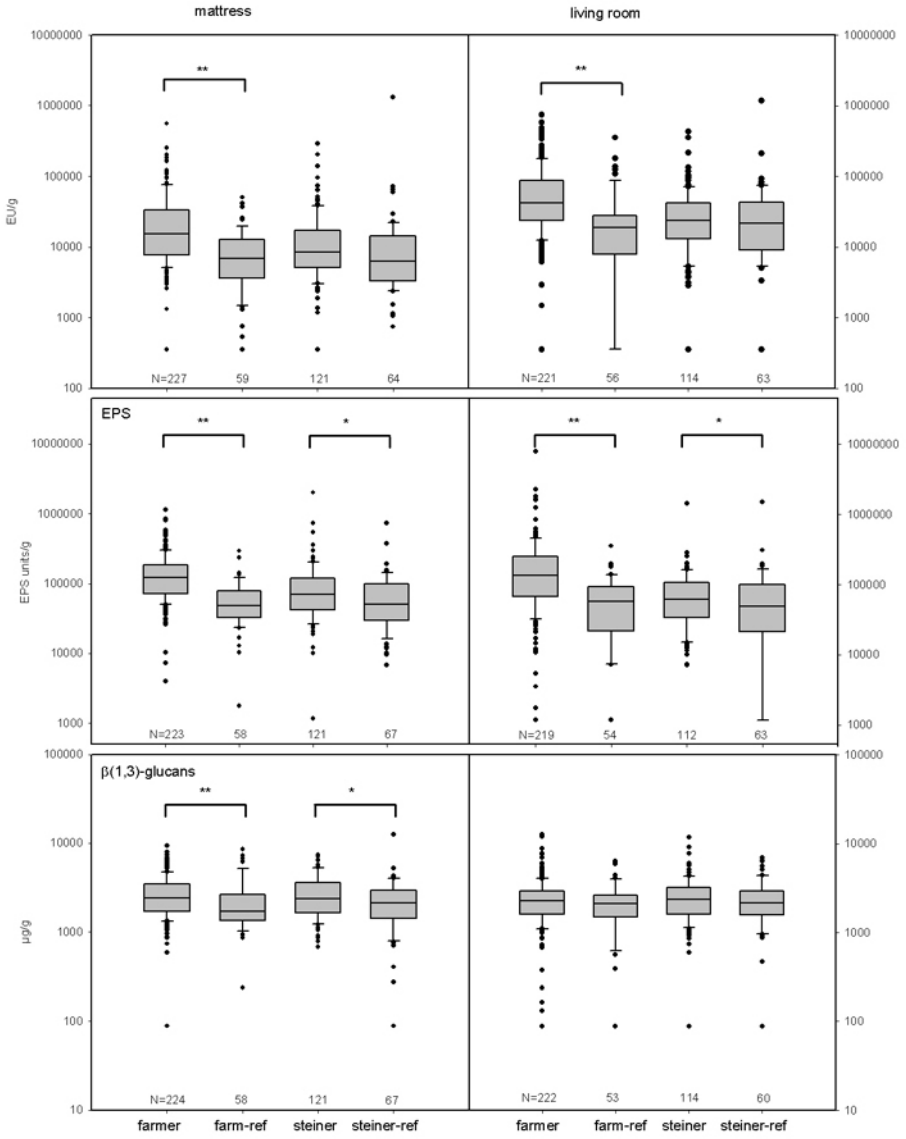

*Figure 1. Boxplots showing medians, 10th, 25th, 75th and 90th percentiles of the levels of microbial components per gram of dust in mattresses and living room floors. *, p < 0.05; **, p < 0.01.*

RESULTS

Differences between groups and countries

More living room and mattress dust was collected in homes of Steiner children as compared to their reference children (GM 0.34 versus 0.17 and 0.45 versus 0.35 g respectively). For farm children, only amounts of living room dust were significantly higher as compared to farm reference children (GM 0.27 versus 0.18 g). Figure 1 shows the distributions of microbial components per gram of dust for both sampling sites for each of the 4 groups. Farm children had higher levels of endotoxin (2.6-3.2 fold), EPS (2.4-3.1 fold) and glucans (1.2-1.3 fold) as compared to farm reference children. Steiner children had somewhat higher levels than their reference children (endotoxin 1.2-1.3 fold.; EPS 1.4-1.6 fold; glucans 1.1-1.2-fold) but differences were smaller and not always significant. For farm children and their reference children, the differences were 1.5-4.2 fold ($p<0.05$) when levels were expressed per square meter, for Steiner children and their reference children, the differences were 1.6-2.5 fold ($p<0.01$) when expressed per square meter (data not shown). The trends were consistent across countries, although mean levels in each country varied considerably (fig. 2). For example, for mattress samples from farm children, mean levels of endotoxin, EPS, and glucans per gram of dust varied up to 2.6, 1.3 and 2.2-fold respectively, between the countries. Highest levels of EPS were observed in Switzerland (mattress) and Austria (living room), whilst highest levels of endotoxin and glucans were observed in Germany and Sweden respectively.

Associations between levels of different microbial agents at the same site and between levels at different sampling sites

For both mattress and living room dust, levels of the three microbial agents per gram dust showed moderate, but significant correlations, with Pearson's r for log-transformed values ranging from 0.20 to 0.38. A comparison of levels in mattress dust with those in living room floor dust from the same home also showed moderate correlations, with r ranging from 0.26 to 0.36.

The levels of endotoxin, EPS and glucans in dust from stables were respectively 5, 7 and 3-fold higher than in dust of living room floors of farm children. EPS and glucans in dust of stables were moderately correlated with the levels of these agents in dust from mattresses and floors with carpets or rugs (r 0.19-0.34), whilst no significant correlations were found for endotoxin (r 0.04-0.06).

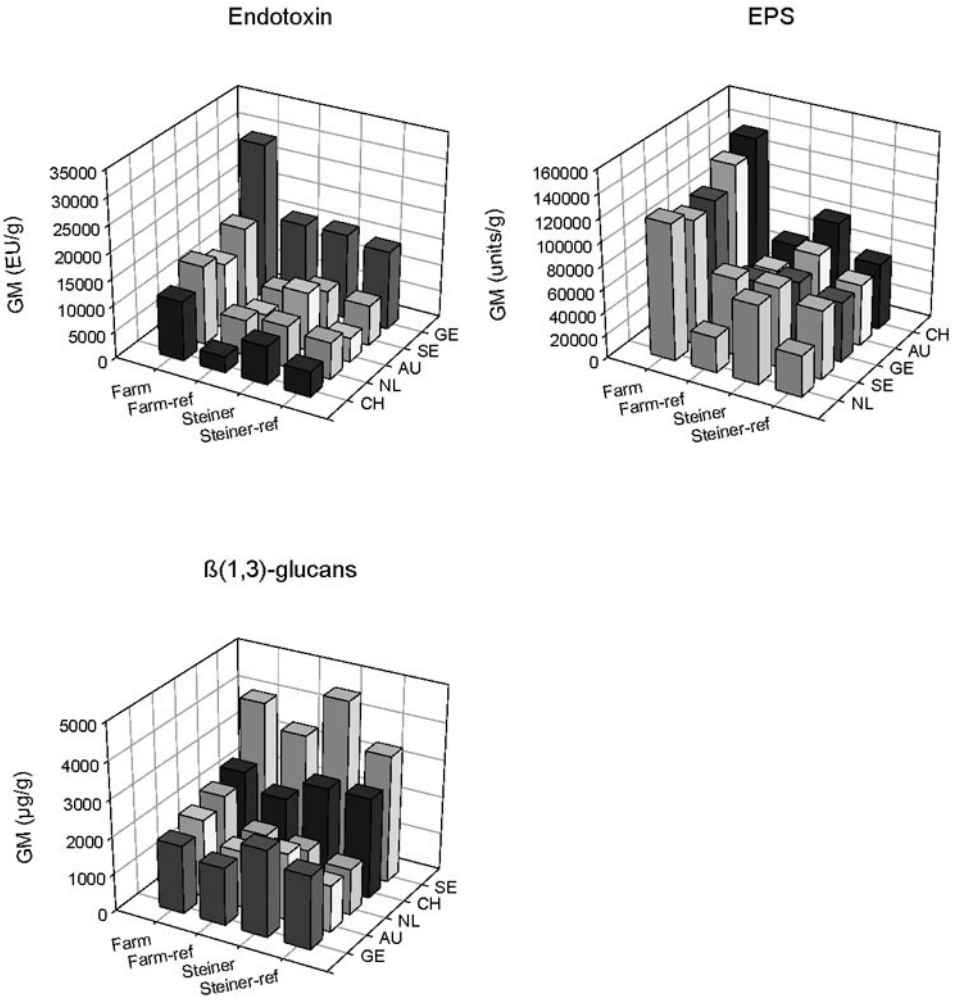

Figure 2. Levels of microbial components in mattress dust by group and by country[a] (GE=Germany, SE=Sweden, AU=Austria, NL=The Netherlands, CH=Switzerland).
a. To improve visibility, results for the different countries are given in a different order in the various graphs.

Table 1. Multivariate association between home and family characteristics and levels of microbial components in mattress dust: means ratio's (MR) and confidence intervals (CI). *, $p < 0.05$; **, $p < 0.01$.

Characteristics	N[a]	Endotoxin (EU/g)	EPS (EPS units/g)	ß(1,3)-glucans (µg/g)
N_{model}		435	433	434
R^2		24.3%	24.8%	33.2%
Group		MR (CI)	MR (CI)	MR (CI)
Farm vs. Steiner-ref[b]	202	1.44 (1.00-2.06)	1.43 (1.08-1.89)*	1.16 (0.97-1.37)
Steiner vs. Steiner-ref	115	1.28 (0.94-1.75)	1.23 (0.97-1.57)	1.18 (1.02-1.37)*
Farm-ref vs. Steiner-ref	54	0.85 (0.59-1.23)	0.88 (0.66-1.18)	0.95 (0.79-1.14)
Country				
Switzerland vs. Sweden	65	0.73 (0.53-1.00)	1.20 (0.93-1.54)	0.66 (0.57-0.78)**
Netherlands vs. Sweden	77	0.99 (0.73-1.35)	0.85 (0.67-1.08)	0.46 (0.39-0.53)**
Germany vs. Sweden	102	2.12 (1.59-2.83)**	1.00 (0.79-1.25)	0.49 (0.43-0.57)**
Austria vs. Sweden	93	0.90 (0.67-1.22)	1.01 (0.80-1.28)	0.46 (0.40-0.53)**
Home and family characteristics[c]				
Dampness or moulds living-/bedroom, yes vs. no	34	1.26 (0.87-1.81)	1.18 (0.89-1.57)	0.99 (0.83-1.18)
House built ≥ 1960 vs. < 1960	204	1.06 (0.86-1.30)	1.25 (1.06-1.47)**	0.98 (0.89-1.09)
Dog vs. no cat and dog	41	1.05 (0.74-1.48)	0.84 (0.64-1.10)	1.02 (0.86-1.20)
Cat vs. no cat and dog	111	1.09 (0.86-1.38)	1.05 (0.87-1.27)	1.11 (0.99-1.25)
Dog and cat vs. no cat and dog	65	1.52 (1.12-2.05)**	1.24 (0.97-1.57)	1.09 (0.94-1.26)
Frequent use of gas/wood stove for heating, yes vs. no	72	0.93 (0.71-1.22)	1.26 (1.01-1.56)*	1.04 (0.91-1.19)
Child's contact to farm animals, yes vs. no	226	1.49 (1.15-1.93)**	1.50 (1.22-1.84)**	1.10 (0.97-1.24)

a. Indicates the number of children with the mentioned characteristic (e.g. 202 farm children, 41 children with a dog etcetera) of 435 children included in the endotoxin model.
b. This model included all 4 groups to increase the power, which implicated that one group had to be chosen as reference group for all groups. We chose Steiner reference children as reference group. The MR for farm vs. farm reference is 1.44/0.85=1.69 for endotoxin, 1.63 for EPS and 1.22 for glucans.
c. Family size, parental education level, age of the mattress, type of floor covering in bedroom and number of days since last vacuuming did not show significant effects and were excluded from the model.

Determinants of microbial agent levels

A number of home characteristics differed between the groups; of the children included in the multivariate analyses described below, more farm children lived in houses built after 1960 (62%) compared to farm reference children (30%). The same was true for Steiner children as compared to Steiner reference children (39% versus 27% respectively). Of the farm children, 88% had frequent contact to farm animals, versus 26% of farm reference children, 23% of Steiner children and 11% of Steiner reference children. Of the farm children, 37% had a carpeted floor or floor with a rug, versus 45% of farm reference children, 59% of Steiner children and 49% of Steiner reference children. We therefore also compared levels of microbial agents between groups, while adjusting for these factors and for country of residence. Table 1 shows the multivariate associations between home and family characteristics and the observed levels in mattress dust. Part of the observed differences between groups was explained by age of the home (EPS), frequent use of a gas or wood stove for heating (EPS), having both a cat and a dog (endotoxin) and contact of the index child to farm animals (EPS and endotoxin). Contact to farm animals was correlated with being a farm child (r=0.67), but the analyses for all groups separately showed that in each group contact to farm animals was associated with higher levels of endotoxin and EPS in mattress dust.

The same multivariate model, including type of floor covering, was used to explain levels per gram of living room dust. Significant, positive associations were found for country, being a farm child (EPS, endotoxin) or Steiner child (EPS), parent-reported dampness or mould growth in the home (EPS), age of the home (EPS), type of floor covering (carpet or rug versus smooth) (EPS, glucans) and frequent use of a gas or wood stove for heating (endotoxin) (data not shown). Table 2 shows the associations between farm characteristics and the observed levels in mattress dust of farm children after adjustment for home and family characteristics and presence of farm animals other than cows and pigs (horses, sheep, chicken, goats). Full time farming increased the levels of EPS in mattress dust. A direct connection between house and stable appeared to increase endotoxin and EPS levels in mattress dust, but these increases were not significant. For living rooms, full time farming was associated with higher levels of EPS and endotoxin (not shown).

Table 2. Multivariate association[a] between farm characteristics and levels of microbial components in mattress dust of farm children: means ratio's and confidence intervals. *, $p < 0.05$; **, $p < 0.01$.

Farm characteristics[b]	N[c]	Endotoxin (EU/g)	EPS (EPS units/g)	ß(1,3)-glucans (µg/g)
N_{model}		178	174	175
R^2		16.8%	19.7%	36.4%
		MR (CI)	MR (CI)	MR (CI)
Full time vs. part time farming	118	0.98 (0.69-1.38)	1.28 (0.99-1.66)	0.88 (0.75-1.04)
House connected to stable, yes vs. no	67	1.27 (0.81-2.01)	1.24 (0.88-1.76)	1.10 (0.88-1.36)
Pig(s) vs. no pig	53	1.01 (0.69-1.49)	0.90 (0.67-1.21)	1.01 (0.84-1.21)
Cow(s) vs. no cow	158	1.27 (0.70-2.29)	0.98 (0.63-1.53)	0.95 (0.72-1.26)

a. Adjusted for country, home and family characteristics (listed in table 1) and the presence of horses, sheep, goats and chicken.
b. Stable visit frequency of the child and number of cows did not show significant effects and were excluded from the model.
c. Of 178 children included in endotoxin model.

DISCUSSION

This study demonstrates that not only endotoxin levels, but also levels of mould EPS and glucans are higher in homes of farm children as compared to homes of farm reference children. Also for Steiner children, somewhat higher levels of endotoxin, EPS and glucans were observed as compared to their reference children, but differences were smaller and not always significant.

Differences between farm children and their reference children

The results regarding endotoxin levels in homes of farm children are in line with the previously published ALEX study which also showed that endotoxin levels in homes of farm children are higher than in homes of reference children in Austria, Germany and Switzerland [7]. We showed exposure differences for mould components as well: EPS and glucans. The differences were most pronounced for EPS, which was previously shown to be a useful marker of indoor mould growth [18]. Glucans are important cell wall constituents of most fungi, but have other sources too, like bacteria and many higher and lower plants. Furthermore, the variation of glucan measurements has been shown to be similar within and between homes, whereas for EPS within-home variation was smaller than that occurring between homes [18]. Although there have been signs that farming also influences the

domestic area [25-27], this is the first study showing elevated indoor exposure to fungal components and a correlation between levels in stable dust and in house dust. It is interesting that such a correlation was not found for endotoxin. In the ALEX study, no correlation between stable endotoxin levels and indoor levels was found either [28]. It might be speculated that endotoxin levels in stables vary more than mould levels over time. This study did not include repeated measurements to assess variability of levels over time, but the ALEX study showed a high correlation (r=0.73) between endotoxin levels in dust samples of stables taken on two separate occasions [28]. It can also be speculated that variation in other sources of indoor levels or variation in factors determining transfer of bacterial and fungal components from stables to the indoor environment cause these apparent discrepancies in associations between levels indoors and in stables.

One important determinant which partly explained the higher endotoxin and EPS levels in mattress dust of farm children was contact to farm animals. Contact to farm animals was also in Steiner children, Steiner reference children and farm reference children associated with higher levels of endotoxin and EPS in mattress dust, although not always significantly. This is in line with the study showing that not only farm children but also non-farm children with regular contact to farm animals are exposed to elevated endotoxin levels [7].

Differences between Steiner children and their reference children

Exposure differences were not only found for farm children and their reference children, but also, though less clearly, for Steiner children and their reference children. More mattress and living room dust was collected in homes of Steiner children as compared to Steiner reference children, which might be related to cleaning habits. Steiner families vacuumed their living room on average 3.4 days before the home visit for the last time, whereas Steiner reference families vacuumed on average 2.4 days before the home visit for the last time (p=0.01), which might indicate that Steiner families clean less often. However, even after adjustment for days since last vacuuming (not shown) and various home characteristics, differences in microbial exposure remained, although the observed differences did not always reach significance. The first studies in this group of children did not include measurements in house dust [21, 29]. Future studies into the factors possibly protecting these children from atopic diseases should take microbial components in house dust into account, although it seems unlikely that the rather small differences in exposure, compared to the differences found between farm children and reference children, would substantially contribute to a presumed protective effect in the development of atopic sensitization, as hypothesized for farm children.

Differences between countries

The differences between groups were consistent across countries. Despite large differences in farming practices, the differences in microbial exposure between farm children and reference children were similar in all countries. The mean levels per country however differed substantially, even after adjustment for differences in home characteristics. This cannot be due to differences in sampling and laboratory procedures, because we used standardized procedures and one laboratory for all analyses. Inter-fieldworker differences might have occurred, but no consistent, significant fieldworker effects on levels of microbial components were observed within countries. There were some differences in the timing of fieldwork, but studies in German homes found no effects of season [30], temperature and relative humidity [30, 31] on endotoxin levels, so this is an unlikely explanation for the observed exposure differences. For EPS and glucans, no seasonal effects have been reported previously either [18, 32]. We have no explanation for the international exposure differences observed in this study.

This study assessed differences in exposure to bacterial and fungal components in house dust between farm and Steiner children and their respective reference children. Exposure to immunomodulatory components of moulds might protect against atopy and asthma, as has been suggested for exposure to endotoxin and other bacterial components [33]. The association between microbial exposure and allergic and airway diseases in these children will be assessed in a separate publication.

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CHAPTER 4

Bacterial and fungal agents in house dust and wheeze in children - the PARSIFAL study

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ABSTRACT

Background Growing up on a farm and an anthroposophic lifestyle are associated with a lower prevalence of allergic diseases in childhood. This might be related to increased inhalatory exposure to microbial agents.
Objective To assess the association between microbial agents in house dust and atopic wheeze in farm children, Steiner school children and reference children.
Methods Levels of bacterial endotoxin, fungal ß(1,3)-glucans and fungal extracellular polysaccharides (EPS) in mattress and living room floor dust were measured in a population of 270 atopic (=Phadiatop-positive) children with self-reported wheezing, including 168 current atopic wheezers, and 441 non-atopic, non-symptomatic controls. These children were selected from a cross-sectional study in 5 European countries.
Results In the study population as a whole, average levels of mattress dust endotoxin, EPS and glucans were slightly (1.1-1.2 fold; $p<0.10$) higher in control children than in atopic wheezers. Atopic wheeze was related to mattress levels of endotoxin, EPS and glucans in farm and farm reference children. However, when adjusting for group (farm versus farm reference children), the associations became non-significant whereas the group effect remained. No associations between atopic wheeze and microbial agents were observed in Steiner and Steiner reference children. For current atopic wheeze, the farm effect became non-significant after adjustment for microbial agent levels.
Conclusion Not only bacterial endotoxin, but also mould components might offer some protection against atopic wheeze in children. However, the protective effect of being raised on a farm was largely unexplained by the mattress microbial agent levels measured in this study.

INTRODUCTION

While the prevalence of asthma and allergy has increased worldwide [1], both diseases appear to be less common in children raised on a farm [2-8] and in children from families with an anthroposophic lifestyle [9]. It has been suggested that early childhood exposure to high levels of microbial components leads to a lower risk of asthma and allergy [10]. The activation of innate immunity mechanisms by these agents may inhibit helper T cell type 2 (T_h2)-type allergic responses [10].

The cross-sectional PARSIFAL (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) study explored factors characteristic of farming and anthroposophic populations that may protect against the development of atopic diseases in children. We recently reported enhanced levels of house dust associated bacterial and fungal agents in farm homes, and to a lesser extent, in

the homes of Steiner school children of that study [11]. We also reported a lower prevalence of sensitization and rhinoconjunctivitis in farm and Steiner children [12] while the prevalence of wheezing was significantly lower in farm children in some countries, but not in others [12]. This study aimed to describe the overall association between reported wheeze and levels of microbial agents in house dust and to investigate whether the lower prevalence of wheeze in farm children can be explained by microbial agent levels. For this purpose, we selected approximately 100 children with self-reported wheeze and 100 children without symptoms from each country of the PARSIFAL population.

Previous studies regarding microbial agents and atopic diseases in children have mainly focused on bacterial endotoxin [13-16]. One study in farm and farm reference children in Austria, Germany and Switzerland showed that endotoxin levels in mattress dust were inversely related to the prevalence of hay fever, allergic asthma, and atopic sensitization. Non-atopic asthma was not significantly associated with endotoxin [15]. However, it has been suggested that protection against asthma and allergy might also result from exposure to other microbial agents [17], such as mould ß(1,3)-glucans, which also have known immunomodulatory effects [18-23], and which we found to be increased in farm homes and homes of Steiner children [11]. We therefore examined the relations between wheeze in children and house dust levels of endotoxin, ß(1,3)-glucans and another marker of fungal exposure [24], extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp*.

METHODS

PARSIFAL study

A total of 14,893 children aged 5-13 years were recruited among farmers (>80% livestock farms), families with an anthroposophic lifestyle, and respective reference groups in five European countries: Austria, Germany, the Netherlands, Sweden, and Switzerland, as described previously [12]. Children with an anthroposophic lifestyle were recruited through Rudolf Steiner schools. Farm reference children were selected such that they lived in the neighborhood of farm children, in rural areas, but did not actually live on a farm. Steiner reference children lived in the neighborhood of Steiner children, mainly in urban areas, but did not attend a Rudolf Steiner school. Questionnaires and consent forms were completed by the parents of the participating children. Allergen-specific immunoglobulin E (IgE) analysis, including Phadiatop (a mix of common inhalant allergens) and fx5 (a mix of common food allergens), was performed centrally [12].

Selection of cases and controls

Atopic and non-atopic children with wheeze and non-atopic, non-symptomatic controls were selected on the basis of IgE analysis and reported symptoms. Children were defined as

wheezers if their parents reported 'wheeze in the past 12 months' or 'wheeze ever' and 'atopic' if they had a positive Phadiatop test. Control children had a negative Phadiatop test and a negative fx5 test and their parents reported that the child had none of the following symptoms: 'wheeze ever', 'asthma ever', 'doctor-diagnosed asthma, bronchitis or pseudocroup ever', 'sneezing or a runny or blocked nose without a cold, ever', 'rhinitis ever', 'itchy rash for at least 6 months, ever' and 'eczema ever'. For this study, we selected all atopic wheezers (total n=270), a random sample of non-atopic wheezers (n=188) and a random sample of non-atopic controls (n=441), by drawing equal numbers of wheezers (circa 100) and controls (circa 100) per country from the population with IgE serology data. The selection was made irrespective of the group (farm, Steiner or reference children) the children belonged to, because we aimed to study whether the protective 'farming' effect could be explained by microbial agent levels; the association between 'group' and atopic wheeze could not have been studied in a selection stratified by group.

Dust collection

House dust from mattresses and living room floors was collected by parents of the participating children. They were sent nylon sampling socks and instructed to vacuum the whole area of the mattress and a square meter of either carpet, smooth floor or rug in the sitting area for 2 minutes according to detailed photo-instructions. After sampling, the socks were returned to the local study center by regular mail, where they were stored at –20 °C. All samples were shipped in frozen conditions to one laboratory (IRAS, Utrecht, NL) for central analysis.

Dust extraction and analysis

Endotoxin, EPS and glucans were extracted sequentially and analyzed as described previously [11]. In short, endotoxin was measured with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, EPS with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium spp.* [25] and glucans with an inhibition EIA [26]. Mite (Der p 1, Der f 1), cat (Fel d 1) and dog (Can f 1) allergens were measured as described previously [27]. Samples with non-detectable amounts (endotoxin: n=23, EPS: 45, glucans: 36) were given a value of two-thirds of the lowest observed detectable amount per gram of dust or per square meter for the specific component determined.

Statistical analysis

Statistical analyses were performed with SAS statistical software (version 8.2, SAS Institute, Cary, N.C.). Levels of microbial agents were approximately normally distributed after natural log (ln)-transformation. Associations with atopic wheeze were investigated for levels of microbial agents per gram dust only, because levels per square meter were considered less reliable; dust was collected by untrained participants with different types of

vacuum cleaners, which might have influenced dust amounts. The relationships between ln-transformed microbial agent levels and atopic wheeze were explored with non-parametric regression by computing smoothed curves, expressing the logit of the rate of atopic wheeze as a continuous function of microbial agent levels, using the generalized additive models procedure of SAS, and were found generally linear. Therefore, logistic regression analyses, in which the microbial agent levels were treated as continuous variables, were performed to calculate odds ratios for an interquartile range increase of each component, with adjustment for country, age and sex. In addition, potential confounding by group (farm, farm reference, Steiner or Steiner reference), parental education, maternal smoking during pregnancy, current smoking in the household, older siblings and allergen levels was evaluated. Models including all groups of children (farm, farm reference, Steiner and Steiner reference), as presented in figure 2, were not adjusted for group because it would be unclear which group should serve as a reference group. However, separate analyses, adjusted for group, were performed for the two branches of the PARSIFAL study; farm/farm reference and Steiner/Steiner reference children (table 3 and 4). The homogeneity of the odds ratios across countries was evaluated by including interaction terms in the models (country*microbial agent levels). In case of heterogeneity ($p<0.10$), combined effect estimates were calculated using random effects estimation [28].

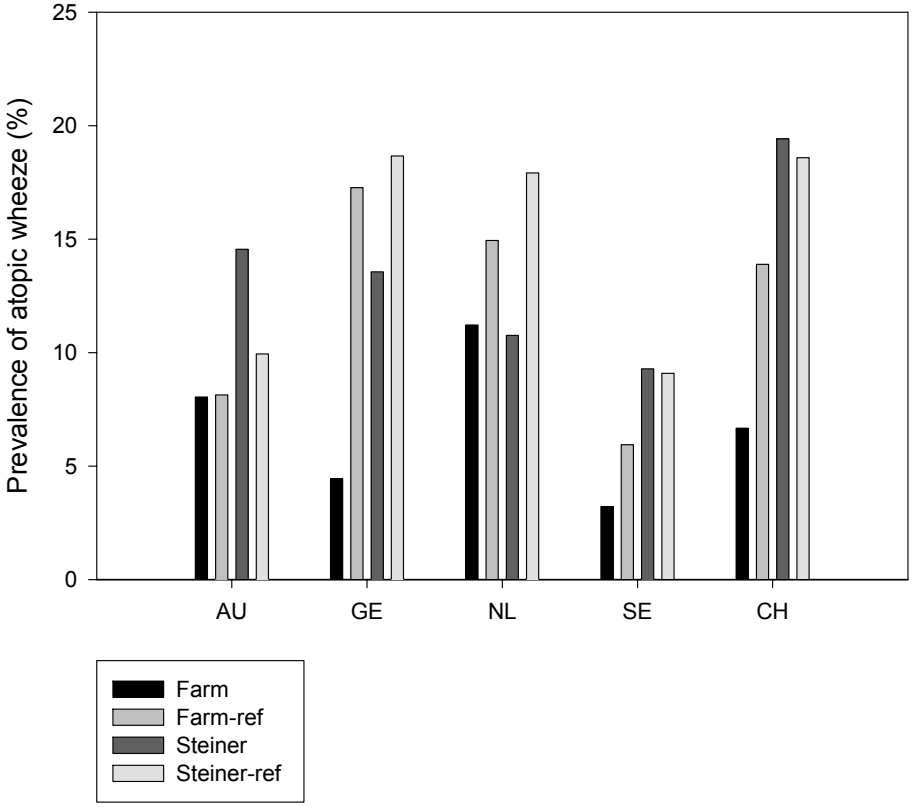

Figure 1. Prevalence of atopic wheeze by group and by country (SE=Sweden, CH=Switzerland, NL=the Netherlands, GE=Germany, AU=Austria).

Table 1. Numbers and percentages of atopic wheezers, non-atopic wheezers and controls by group and by country; approximately 100 children with self-reported wheeze and 100 non-atopic, non-symptomatic children were selected per country from the population with IgE serology data.

	Source population	Analysis population	Atopic wheezers	Non-atopic wheezers	Controls
	N	N	N (%)	N (%)	N (%)
Austria	*757*	*186*	*49*	*47*	*90*
Farm	214	50	10 (20.0)	11 (22.0)	29 (58.0)
Steiner	233	63	21 (33.3)	20 (31.7)	22 (34.9)
Farm-ref	135	29	6 (20.7)	6 (20.7)	17 (58.6)
Steiner-ref	175	44	12 (27.3)	10 (22.7)	22 (50.0)
Germany	*1154*	*182*	*57*	*39*	*86*
Farm	623	80	12 (15.0)	14 (17.5)	54 (67.5)
Steiner	245	43	18 (41.9)	11 (25.6)	14 (32.6)
Farm-ref	147	43	20 (46.5)	10 (23.3)	13 (30.2)
Steiner-ref	139	16	7 (43.8)	4 (25.0)	5 (31.3)
Netherlands	*552*	*167*	*35*	*47*	*85*
Farm	102	26	4 (15.4)	9 (34.6)	13 (50.0)
Steiner	162	59	11 (18.6)	11 (18.6)	37 (62.7)
Farm-ref	181	53	11 (20.8)	18 (34.0)	24 (45.3)
Steiner-ref	107	29	9 (31.0)	9 (31.0)	11 (37.9)
Sweden	*833*	*184*	*46*	*49*	*89*
Farm	253	61	7 (11.5)	19 (31.1)	35 (57.4)
Steiner	353	83	27 (32.5)	18 (21.7)	38 (45.8)
Farm-ref	105	19	3 (15.8)	6 (31.6)	10 (52.6)
Steiner-ref	122	21	9 (42.9)	6 (28.6)	6 (28.6)
Switzerland	*743*	*180*	*83*	*6*	*91*
Farm	222	47	9 (19.1)	1 (2.1)	37 (78.7)
Steiner	252	64	38 (59.4)	3 (4.7)	23 (35.9)
Farm-ref	150	41	19 (46.3)	1 (2.4)	21 (51.2)
Steiner-ref	119	28	17 (60.7)	1 (3.6)	10 (35.7)
Total	*4039*	*899*	*270*	*188*	*441*

Table 2. Subject characteristics and microbial agent levels in mattress dust (ns=not significant: $p > 0.1$).

	Atopic wheezers N=270	Controls N=441	p-value
Age, mean (SD)	9.0 (1.8)	8.8 (1.8)	ns
Male (%)	69.3	46.5	< 0.0001
Maternal asthma and/or rhinoconjunctivitis (%)	34.5	15.4	< 0.0001
Paternal asthma and/or rhinoconjunctivitis (%)	32.0	15.3	< 0.0001
High parental education (%)	42.3	36.6	ns
Smoking mother during pregnancy (%)	10.9	6.0	0.02
Current smoking in the household (%)	12.0	12.5	ns
Older siblings (%)	65.8	67.6	ns
Microbial agents per gram dust			
Endotoxin (EU/g), GM (GSD)	22,268 (3.06)	26,498 (2.95)	0.05
EPS (EPS units/g), GM (GSD)	58,814 (2.85)	69,139 (3.46)	0.07
ß(1,3)-glucans (µg/g), GM (GSD)	2,662 (2.12)	2,959 (1.94)	0.07
Microbial agents per square meter			
Endotoxin (EU/m^2), GM (GSD)	3,396 (4.81)	4,570 (4.50)	0.01
EPS (EPS units/m^2), GM (GSD)	8,511 (6.34)	12,148 (6.78)	0.02
ß(1,3)-glucans (µg/m^2), GM (GSD)	402 (4.60)	519 (3.98)	0.03

RESULTS

Figure 1 shows the differences in atopic wheeze prevalence between farm and farm reference children and Steiner and Steiner reference children by country, in the population with IgE serology, from which we selected our cases and controls. In the German and Swiss population, farm children had a significantly lower prevalence of atopic wheeze than farm reference children, whereas the differences were not significant in the other countries. A lower prevalence of atopic wheeze in Steiner children as compared to Steiner reference children was only observed in the Netherlands.

Characteristics of the selected population

Table 1 shows the numbers and group compositions for the cases and controls selected for this study by country. By design, the total number of controls was nearly equal in each country. Although the total number of wheezers was nearly equal as well, the numbers of atopic and non-atopic wheezers were different across countries, as all atopic wheezers were included. In addition, percentages of farm, Steiner and reference children among cases and controls differed across countries due to the differences in size of the groups in the population with IgE serology (column 1 of table 1) and group-specific prevalence rates of atopic wheeze (fig. 1). For example, the German analysis population contained relatively many farm children, because of the large number of farm children in the population with IgE serology. Since the German farm children also showed a very low prevalence of atopic wheeze, controls from Germany largely consisted of farm children (table 1). Non-atopic wheezers were not included in further analyses because of the low prevalence of 'wheeze in the past 12 months' in this group (20% versus 63% in the atopic wheeze group).

Table 2 shows demographic characteristics and microbial agent levels per gram mattress dust of cases and controls. The subject characteristics of this analysis population were essentially similar to the characteristics of atopic wheezers and controls in the total population with IgE serology (n=4,039) (not shown). Geometric mean levels of endotoxin, EPS and glucans per gram mattress dust were 1.1-1.2 fold higher in control children than in atopic wheezers. Levels in living room floor dust were also higher in control children than in atopic wheezers, though significantly only for levels per square meter. Further analyses have been restricted to mattress levels. Correlations between levels per gram mattress dust of allergens and microbial agents, between the microbial agents, and between group and microbial agents, were generally low (r for log transformed data < 0.41). Therefore, their independent effects could be assessed in multivariate analyses.

Associations between microbial agent levels and atopic wheeze

Figure 2 shows the odds ratios for endotoxin, EPS and glucans in mattress dust and atopic wheeze by country. Heterogeneity was observed for endotoxin (p=0.07); levels were inversely related to atopic wheeze in three countries, particularly in Germany, but for Sweden the association was in the other direction. The odds ratios for glucans and EPS were more similar across countries and overall odds ratios were borderline significant. Similar results were obtained when allergens were included in the models (not shown).

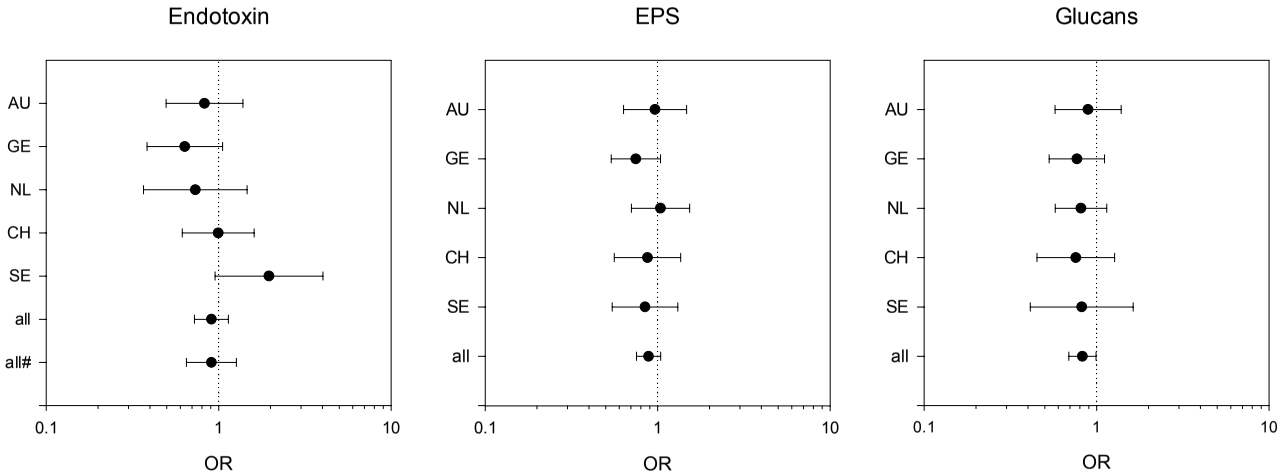

Figure 2. Odds ratio's and 95% confidence intervals for atopic wheeze with an interquartile range increase of each microbial agent per gram mattress dust, adjusted for country, age, sex, older siblings, parental education level, smoking in the household and smoking mother during pregnancy (SE=Sweden, CH=Switzerland, NL=the Netherlands, GE=Germany, AU=Austria).
 # Odds ratio based on meta-analysis using random effects estimation because of heterogeneity.

Table 3. Odds ratio's and 95% confidence intervals for atopic wheeze with an interquartile range increase of each component per gram mattress dust[a].

	AOR[b]	Mutually adjusted AOR[c]
Farm and farm reference children (N[d]=329)		
Farm vs. farm reference children	0.34 (0.20-0.56)	0.36 (0.20-0.64)
Endotoxin	0.71 (0.52-0.97)	1.03 (0.69-1.55)
EPS	0.79 (0.63-0.98)	0.95 (0.70-1.30)
Glucans	0.77 (0.58-1.01)	0.83 (0.56-1.22)
Steiner and Steiner reference children (N[d]=334)		
Steiner vs. Steiner reference children	0.86 (0.53-1.41)	0.89 (0.52-1.51)
Endotoxin	1.09 (0.78-1.53)	1.22 (0.84-1.77)
EPS	1.02 (0.82-1.28)	0.96 (0.74-1.25)
Glucans	0.90 (0.71-1.12)	0.92 (0.66-1.28)

a. A factor 4.0 for endotoxin, 3.0 for EPS and 1.9 for glucans in all models, based on the interquartile ranges in the total population. Results were essentially the same when using group-specific interquartile ranges.
b. Adjusted for country, age, sex.
c. Adjusted for group, microbial agents and the factors mentioned under b.
d. Number of children in mutually adjusted model.

Table 4. Odds ratio's and 95% confidence intervals for **current** atopic wheeze with an interquartile range increase of each component per gram mattress dust[a].

	AOR[b]	Mutually adjusted AOR[c]
Farm and farm reference children (N[d]=287)		
Farm vs. farm reference children	0.45 (0.24-0.86)	0.58 (0.29-1.19)
Endotoxin	0.74 (0.51-1.08)	1.09 (0.66-1.78)
EPS	0.74 (0.57-0.95)	0.77 (0.54-1.10)
Glucans	0.78 (0.57-1.07)	0.91 (0.58-1.43)
Steiner and Steiner reference children (N[d]=283)		
Steiner vs. Steiner reference children	0.76 (0.44-1.31)	0.81 (0.45-1.45)
Endotoxin	1.20 (0.82-1.74)	1.38 (0.92-2.09)
EPS	0.94 (0.74-1.19)	0.89 (0.67-1.18)
Glucans	0.89 (0.68-1.16)	0.80 (0.56-1.16)

a. A factor 4.0 for endotoxin, 3.0 for EPS and 1.9 for glucans in all models, based on the interquartile ranges in the total population.
b. Adjusted for country, age, sex.
c. Adjusted for group, microbial agents and the factors mentioned under b.
d. Number of children in mutually adjusted model.

Separate analyses were performed for the two branches of the PARSIFAL study; farm children and references and Steiner children and references (table 3). Protective effects of endotoxin, EPS and glucans in mattress dust were observed for farm and farm reference children, but when adjusting for group, the effects became non-significant. The effect of group on the other hand did not change by including microbial agents in the model. For Steiner and Steiner reference children, no significant effects of group and microbial agent levels were observed. Results were essentially the same after adjustment for potentially confounding factors, like parental education, maternal smoking during pregnancy, current smoking in the household and older siblings.

We performed sensitivity analyses by exluding children who did not report wheeze in the last 12 months (table 4). In farm and farm reference children, the protective farm effect appeared to become smaller and non-significant when microbial agent levels were included into the model. The farm effect was, however, still largely unexplained. For Steiner- and Steiner reference children, no significant effects of group and microbial agent levels to current atopic wheeze were observed.

DISCUSSION

This study demonstrated that microbial agent levels in house dust were related to atopic wheeze in farm and farm reference children. After adjustment for living on a farm however, the effects became non-significant. For current atopic wheeze, the 'farm-effect' became smaller and non-significant after adjustment for mattress microbial agent levels but the 'farm-effect' was still largely unexplained.

This cross-sectional study is based on house dust analyses in a large number of children with wheeze and controls from 5 different countries and different subgroups; farm and Steiner children and their respective references. Despite differences across subgroups and across countries, indications of a protective effect of mould components to atopic wheeze in farm and farm reference children were observed, which have not been reported previously. Whether these effects reflect a down-regulation of the development of atopy and/or the development of wheeze cannot be determined on the basis of our data, neither could we determine whether timing of exposure plays a role; it is an area in which further exploration is needed.

Exposure to microbial agents was assessed by analyses of house dust collected by parents of the participating children. Dust amounts might have been influenced by variation in sampling time or sampling area, although parents were instructed to follow standard procedures. In addition, differing types of vacuum cleaners may have influenced dust amounts. A previous study showed high correlations between allergen levels of subject- and fieldworker-collected dust samples, but subjects collected lighter dust samples

[29]. The validity of the methods of microbial exposure assessment applied in this study, using dust 'socks', collected by participants, will be described in a separate publication. In this study, associations with wheeze were calculated for levels per gram dust only, to factor out variation in dust amounts.

Our results are in line with the previously published ALEX study [15], which showed an odds ratio of 0.89 (0.57 – 1.39) for the occurrence of atopic wheeze with an increase in the endotoxin level per gram mattress dust from the lowest to the highest quartile. When restricting the analyses to similar groups of children within the PARSIFAL study; farm and farm reference children in Germany, Austria and Switzerland, and without adjustment for group (which was not adjusted for in ALEX), we observed an adjusted odds ratio (aOR) of 0.56 (0.35-0.90). For levels per square meter, the aOR was 0.72 (0.49-1.06) versus 0.62 (0.39-0.99) in ALEX. We showed however, that after adjustment for group or glucans and EPS, the effect of endotoxin was not significant. Exposures to endotoxin, EPS and glucans were moderately, but significantly correlated, precluding a firm conclusion on the degree to which specific agents contributed to the observed effect. Moreover, these microbial agents might be markers of a much broader spectrum of microbial agents. However, our results suggest that mould components might also modulate immune responses and thereby protect against allergic diseases, as previously suggested for endotoxin [13-15]. This is in line with a study on the effects of endotoxin and fungal spores on atopy and asthma in adult farmers, in which fungal spores, rather than endotoxin, were inversely related to atopic wheeze [30].

The protective effects of microbial agents were mainly observed in farm and farm reference children. This might be because the total inhalatory exposure to microbial agents is much higher in these children than in Steiner and Steiner reference children, as they are not only exposed to microbial agents in house dust, but probably also to microbial agents in stable dust. We previously showed that levels of microbial agents in stable dust were 3 to 7 fold higher than levels in house dust. We also showed that indoor levels of EPS and glucans were significantly correlated with levels in stable dust [11], while for endotoxin no significant relation could be found. EPS and glucans in mattress dust might be better markers of total inhalatory exposure to several microbial components because of this correlation between levels in stables and levels indoors.

Mattress glucan and EPS levels explained part of the protective 'farming' effect on atopic wheeze, but only when analyses were restricted to wheeze in the last 12 months. The total group of wheezers included children with transient wheeze, which might represent developmental airway abnormalities, resulting in wheeze with viral respiratory infections [31]. The protective effects of microbial agents were more pronounced in the small group of atopic children with severe wheeze (4 or more attacks in the last 12 months, n=51, not shown), indicating that the results may have been biased towards the null by including a mixture of wheezing phenotypes. Another explanation for the stronger association between

microbial agents and current atopic wheeze as compared to wheeze ever is that house dust analyses and current atopic wheeze refer to the same time window. However, even with these confined definitions of atopic wheeze, the farming effect was not completely explained by microbial agent levels in mattress dust. This is in line with the ALEX study, which demonstrated that independent of endotoxin levels in mattress dust 'exposure to farming during the first year' (exposure to stables and/or consumption of milk directly from the farm) showed a protective effect on atopic wheeze [15]. The associations between these and other farm-related factors and allergic disease in the PARSIFAL population will be described elsewhere. The effects of microbial agents on current atopic wheeze as described in this paper could, however, not be explained by contact to pets or farm animals, stable visits or drinking milk directly from a farm (not shown).

Our selections of cases and controls was made irrespective of the groups the children originally belonged to, which resulted in an unbalanced group composition across countries. We therefore adjusted our analyses for group and country, and – in case of heterogeneity across countries, like for endotoxin – by calculating an overall odds ratio with meta-analysis techniques (i.e. random effects estimation). The numbers of cases were too low for stratification by group and by country. However, adjustment for group may have resulted in an underestimation of the effects of microbial components on atopic wheeze, because group was shown to be a determinant of mattress microbial agent levels [11]. Moreover, measurement of 'group' is less subject to measurement error when compared to measurement of microbial agent levels, which might explain why group was a stronger determinant of atopic wheeze.

Our results suggest that microbial agent levels, especially mould components, in house dust may offer some protection against atopic wheeze in children. However, the protective effect of growing up on a farm was largely unexplained by the mattress microbial agent levels measured in this study.

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CHAPTER 5

A non-linear relation between mite allergen levels and sensitization in children from 5 countries

Schram-Bijkerk, D., Doekes, G., Boeve, M., Douwes, J., Riedler, J., Üblagger, E., von Mutius, E., Budde, J., Pershagen, G., Van Hage, M., Wickman, M., Braun-Fahrländer, C., Waser, M., Brunekreef, B. and the PARSIFAL study group

ABSTRACT

Background Low sensitization rates to common allergens have been observed in children from farming and anthroposophic populations. Little is known about the association between specific allergen exposure and sensitization and potential modifying effects of microbial agents, in these populations.

Objective To examine the relations between house dust mite allergen exposure and mite sensitization in farm, Steiner school and reference children and to assess the effects of microbial agents levels on this association.

Methods Major mite allergens of *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1), bacterial endotoxin, fungal ß(1,3)-glucans and fungal extracellular polysaccharides (EPS) were measured in mattress dust of 402 children participating in a cross-sectional study in 5 European countries. Allergen levels (Der p 1 + Der f 1) were divided into tertiles with cut-offs 1.4 and 10.4 µg/g. Sensitization was assessed by measurement of allergen-specific IgE against house dust mite.

Results Prevalence ratios of mite sensitization for medium and high as compared to low mite allergen levels were 3.1 [1.7-5.7] and 1.4 [0.7-2.8] respectively. Highest mite sensitization rates at intermediate exposure levels were consistently observed across country (except for Sweden) and farm, Steiner school and reference children. The shape of the dose-response curve was similar for above and below median mattress microbial agent levels, but the 'sensitization peak' was lower for above median levels.

Conclusion Our data suggest a bell-shaped dose-response relationship between mite allergen exposure and sensitization to mite allergens and support the hypothesis that high levels of microbial agents might protect against sensitization.

INTRODUCTION

Several studies have shown a dose-response relationship between house dust mite allergen exposure and sensitization to house dust mites [1-4]. High levels of house dust mites and their allergens have been observed in farm homes [5, 6], whereas sensitization to mite allergens in adult farmers was similar or lower than the prevalence in the general population [5, 7]. Specific protective factors of the farming environment, such as elevated exposure to bacterial endotoxin [8], might account for this discrepancy. Previous studies have shown a protective effect of endotoxin to sensitization [9, 10], but since the association between allergen levels and allergen-specific sensitization was not investigated in those studies, it is unknown in which way microbial agents, like endotoxin, modify the dose-response relationship.

The PARSIFAL study (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) is a cross-sectional survey, conducted in 5 European countries; Sweden, Switzerland, Germany, Austria and the Netherlands. The aim of the study was to identify factors associated with farming and an anthroposophic lifestyle that confer protection against the development of atopic diseases in children. Children of families with an anthroposophic lifestyle ('Steiner school children') were also included in this study, because in these children, like in farm children, a lower prevalence of atopic diseases was observed previously [11]. We recently reported that levels of bacterial and fungal agents in house dust were increased in farm homes, and to a lesser extent in homes of Steiner school children in the PARSIFAL study [12]. We also reported a lower prevalence of atopic sensitization to common inhalant allergens in farm (OR 0.53 (0.42-0.67)) and Steiner school children (OR 0.73 (0.58-0.92)) when compared to reference children [13]. However, relations between allergen levels and allergen-specific sensitization rates were not addressed in these papers.

In the present study, we investigated 1) whether in the PARSIFAL population dose-response relations could be demonstrated between mite allergen levels in house dust and mite sensitization 2) whether the relation was different in the various subgroups; farm, Steiner and reference children and 3) in which way microbial agents in house dust modified this relationship. For this purpose, we studied the effects of bacterial endotoxin and fungal ß(1,3)-glucans, both of which have strong immunomodulatory properties [14-20], and extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp.*, a general marker of fungal exposure [21].

METHODS

Study population

The PARSIFAL study included a total of 14,893 children aged 5-13 years from farmers', anthroposophic and respective reference families in 5 European countries, as described previously [13]. Shortly, farm children lived on a farm, Steiner children attended a Rudolf Steiner school and references lived near these children, but did not live on a farm or did not attend such a school respectively. Questionnaires were completed by their parents. Allergen-specific immunoglobulin E (IgE) was assessed in a random sample of children whose parents gave consent (n=4,039), by Phadiatop (a mix of common inhalant allergens) and fx5 (a mix of common food allergens). Phadiatop-positive samples were further tested for IgE to specific allergens (listed in table 1) and defined positive if IgE values were ≥ 0.35 kU/L. House dust was collected from mattresses and living room floors in a randomly selected population of 229 children from livestock farms, 122 Steiner children and 60 and

67 of their respective references, with nearly equal numbers per country, as described previously [12]. For 402 (84%) of these children, IgE analyses were available.

Dust extraction and analysis

Endotoxin, EPS, pet and mite allergens, and glucans were extracted from the dust as described previously [12]. Endotoxin was measured in the extracts with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, EPS with a specific sandwich Enzyme Immuno Assay (EIA) for EPS of *Aspergillus* and *Penicillium spp.* [22] and ß(1,3)-glucans with an inhibition EIA [23]. Mite allergens of *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) and the major cat allergen (Fel d 1) were measured with sandwich EIAs as described previously [24]. The average inter-day/inter-assay coefficients of variation, as determined by testing duplicate extract aliquots of 10% of all samples on another day as the first aliquot, ranged from 14.5-30.5%. Results of repeated dust sampling within the framework of PARSIFAL will be described in a separate paper, but showed reasonably good correlations between multiple dust samples taken in the same home a few days to several months apart, for levels of allergens and microbial agents.

Few samples (<4%) had levels of microbial agents or cat allergen below the limit of detection, Der p 1 and Der f 1 could not be detected in 103 and 79 of 395 mattress samples (ca. 65% samples from Sweden) respectively. Non-detectable samples were given a value of two-thirds of the lowest observed detectable amount. Mite allergen exposure (Der 1) was calculated as the sum of Der p 1 and Der f 1.

Statistical analysis

Differences between groups were evaluated by performing non-parametric Wilcoxon tests (allergen levels) and Chi square tests (sensitization rates), using SAS statistical software (version 8.2, SAS Institute, Cary, N.C.). Mite allergen levels (Der 1) were categorized into three categories, based on tertiles. Prevalence ratios for mite sensitization, with the lowest exposure category as reference, were calculated with PROC GENMOD of SAS, with adjustment for age, sex and group, and -in subsequent analyses- country. Mattress microbial agent levels were categorized into above and below median exposure. Smoothed dose-response curves between mite allergen levels and mite sensitization, stratified by dichotomized microbial agent levels, were computed using the generalized additive models procedure of SAS. To test effect-modification by microbial agent levels significance of the interaction variable (categorized mite*categorized microbial agent level) was evaluated with PROC GENMOD.

RESULTS

Table 1 shows mattress mite and cat allergen levels and sensitization rates for farm children, Steiner children and their references. Der p 1 levels were 4-5 times higher in farm children when compared to reference children. In contrast, Der f 1 levels were significantly lower in farm children when compared to reference children. Interestingly, rural farm reference children also showed lower Der f 1 levels when compared to urban Steiner and Steiner reference children. The sum of both mite allergens (Der 1) however, was in the same order of magnitude for all groups. Median Der 1 levels per square meter ranged from 518 ng/m^2 in farm reference children to 1,058 ng/m^2 in farm children and did not significantly differ between farm or Steiner children and reference children either (not shown). Because of similar Der 1 levels, and because of relatively low numbers, the groups of farm reference and Steiner reference children were combined in subsequent analyses. Cat allergen levels were significantly higher in farm and Steiner children when compared to reference children (table 1).

Compared to reference children, farm children were less likely to be sensitized to all tested allergens, except for storage mite, although the difference was not statistically significant for cat allergens and borderline significant for mite allergens and tree pollen (table 1). Sensitization rates were also lower in Steiner children when compared to references, but differences were, except for grass pollen, less pronounced. Prevalences of storage mite sensitization were low and similar across groups. Sensitization rates and differences between groups in this subpopulation with dust analyses were similar to rates and differences in the total population with IgE serology (n=4,039); for example, mite sensitization rates were 10.1%, 17.3%, 18.7% and 20.9% for the total group of farm, farm reference, Steiner and Steiner reference children respectively. Differences between groups were also observed after adjustment for potential confounders such as country, age, sex, parental education, maternal smoking during pregnancy, current smoking in the household, older siblings, mother's reported asthma and/or rhinoconjunctivitis and father's reported asthma and/or rhinoconjunctivitis (not shown). Thus, despite high cat allergen levels, low or similar cat sensitization rates were observed in farm and Steiner children as compared to references, and farm and Steiner children also had a low prevalence of mite sensitization despite the fact that they had similar exposures to mite allergen levels as compared to references. The association between cat allergen levels and cat sensitization was not further studied because of low numbers of cat-sensitized children.

Table 1. Medians and 25th and 75th percentiles of allergen levels per gram mattress dust and sensitization rates by group. #, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$ for farm and Steiner children versus combined group of reference children.

	Farm N=197	Farm-ref N=50	Steiner N=99	Steiner-ref N=56
Mattress allergen levels (ng/g)	Median (p25-p75)	Median (p25-p75)	Median (p25-p75)	Median (p25-p75)
Der p 1	1,591 ** (124-11,858)	293 (20-1,706)	462 (20-3,442)	377 (20-1,891)
Der f 1	226 ** (36-1,132)	950 (14-5,738)	1,969 (322-9,311)	2,740 (314-9,653)
Der p 1 + Der f 1	3,660 (411-15,865)	2,465 (307-8,779)	5,352 (820-13,391)	3,628 (650-13,197)
Fel d 1	6,822 * (1,909-43,889)	1,729 (448-110,049)	2,617 # (977-78,193)	1,621 (607-28,388)
Sensitization	N (%)	N (%)	N (%)	N (%)
Phadiatop-pos	47 (23.9) **	18 (36.0)	30 (30.3)	23 (41.1)
Cat	9 (4.6)	3 (6.0)	8 (8.1)	5 (8.9)
House dust mite[a]	27 (13.7) #	10 (20.0)	16 (16.2)	13 (23.2)
Grass pollen	29 (14.7) **	15 (30.0)	15 (15.2) *	17 (30.4)
Tree pollen	24 (12.2) #	11 (22.0)	11 (11.1) #	10 (17.9)
Storage mite[b]	11 (5.6)	0	5 (5.1)	3 (5.4)

a. Dermatophagoides pteronyssinus
b. Lepidoglyphus destructor

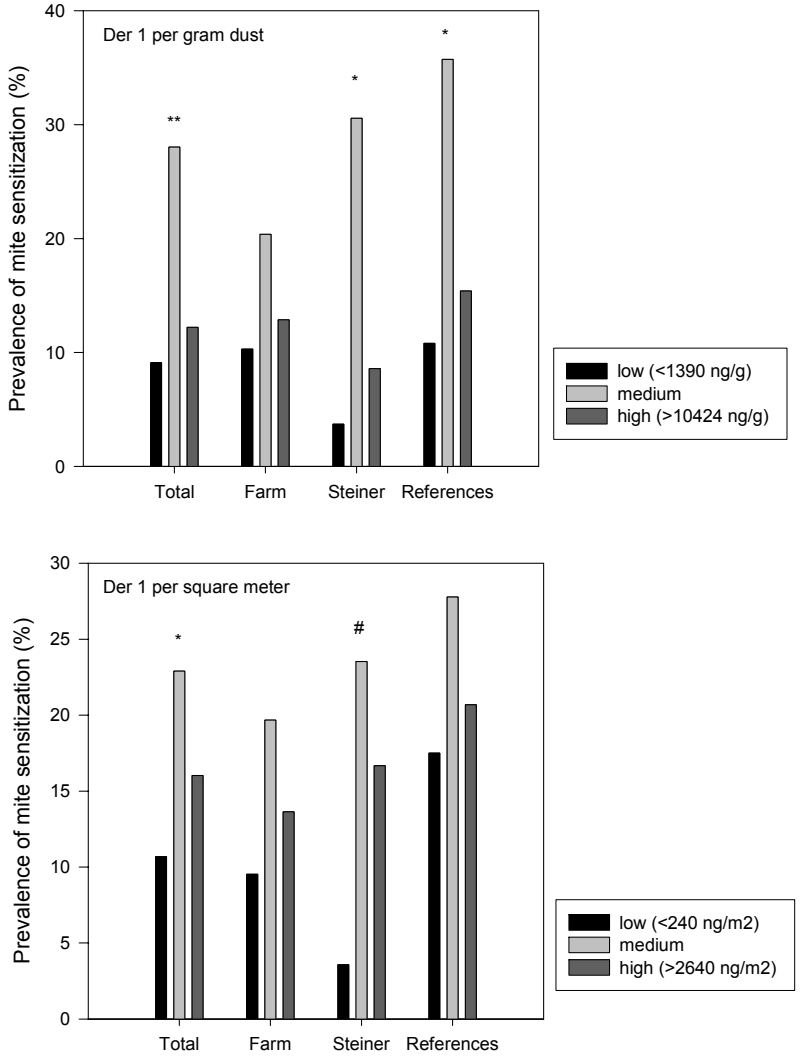

*Figure 1. Prevalence of mite sensitization by tertiles of mattress Der 1 levels per gram dust and per square meter. #, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$ as compared to low exposure.*

Figure 1 shows the prevalence of mite sensitization by tertiles of mattress mite allergen levels per gram dust (cut-offs 1,390 and 10,424 ng/g) and per square meter (cut-offs 240 and 2,640 ng/m^2). Highest sensitization rates were observed at intermediate exposure levels. This was consistent across farm, Steiner and reference children. However, the absolute prevalence rates were lower for farm children when compared to Steiner children and reference children. Similar results were obtained for sensitization rates by tertiles of mite allergen levels expressed per square meter; though differences in prevalences across mite allergen categories were somewhat smaller. Categories of mite allergen levels per gram dust agreed well with categories per square meter (kappa 0.72) and results of subsequent analyses will therefore be presented for allergen levels expressed per gram dust only.

Table 2. Number of mite sensitized children by tertiles of Der 1 levels per gram mattress dust by country.

$N_{sensitized}/N_{category}$ Percentage	Low (<1,390 ng/g)	Medium	High (>10,424 ng/g)
Overall, N=395	12/132	37/132	16/131
	9.1%	28.0%	12.2%
Sweden, N=94	2/81	0/5	0/8
	2.5%	0%	0%
Switzerland, N=45	1/8	4/19	1/18
	12.5%	21.1%	5.6%
Netherlands, N=76	2/12	10/40	5/24
	16.7%	25.0%	20.8%
Germany, N=94	4/20	12/44	3/30
	20.0%	27.3%	10.0%
Austria, N=86	3/11	11/24	7/51
	27.3%	45.8%	13.7%

Table 2 shows the number of mite-sensitized children by tertiles of mite allergen levels by country. Highest sensitization rates at intermediate allergen levels were observed for each country, except Sweden. The majority of Swedish mattress samples (86%) had low mite allergen levels and only 2 children were sensitized to house dust mites. Similar results were obtained when using cut-off levels based on tertiles of the distribution of mite allergen levels in the mite-sensitized population (2,954 and 7,972 ng/g) or after adjustment for age, sex, parental education, maternal smoking during pregnancy, current smoking in the household, older siblings, mother's reported asthma and/or rhinoconjunctivitis and father's reported asthma and/or rhinoconjunctivitis (not shown).

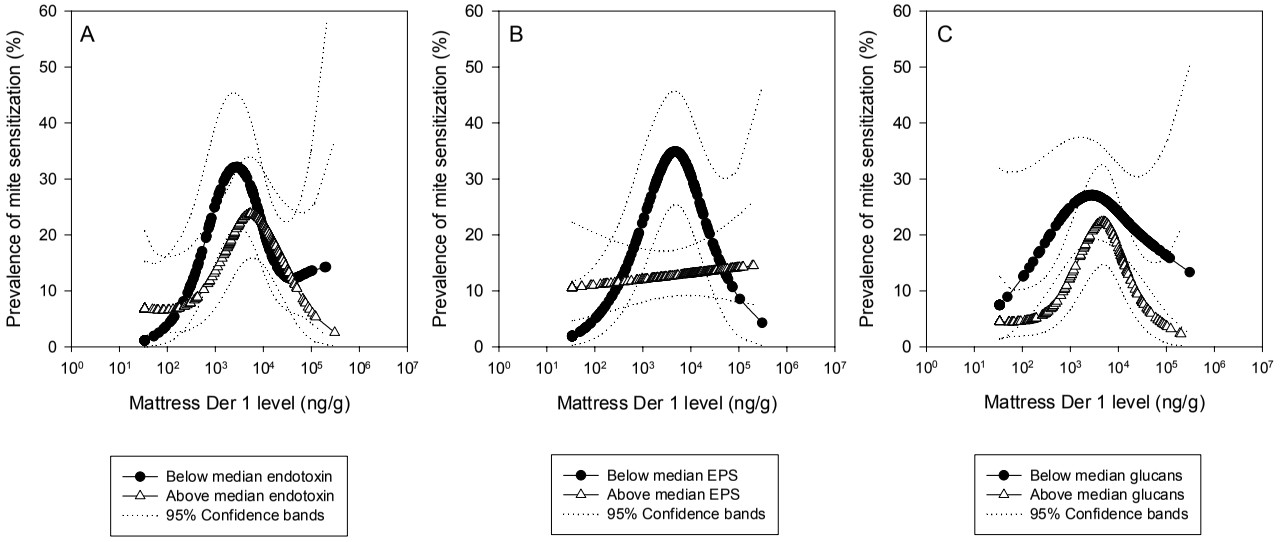

Figure 2. Smoothed plots of the prevalence of mite sensitization in relation to mite allergen levels, stratified by a) dichotomized endotoxin levels b) dichotomized EPS levels and c) dichotomized glucan levels in mattress dust. Smoothness of the plots was determined by generalized cross validation.

When children whose parents reported any measures to reduce allergen levels (n=46) were excluded, prevalences of mite sensitization were 9.4, 31.5 and 12.2% at low, intermediate and high exposure levels respectively. In children whose parents did not report asthma and/or rhinoconjunctivitis (n=235), prevalences of mite sensitization were 8.6, 17.8 and 6.2% at low, intermediate and high exposure levels, respectively as compared to 10.2, 41.4 and 21.3% in children with parental asthma and/or rhinoconjunctivitis (n=154). Also for Der p 1 and Der f 1 levels separately, highest sensitization rates were observed at intermediate mite allergen levels.

To study the dose-response relationship between mite allergen levels and mite sensitization independent of cut-off levels and to study the effects of microbial agents on this relationship, smoothed dose-response curves stratified by dichotomized microbial agent levels were plotted (fig. 2). The effects of microbial agent levels were also studied by multivariate regression analysis (table 3).

Table 3. Prevalence ratios (PR) with 95% confidence intervals (CI) of mite sensitization for medium and high mattress Der 1 levels as compared to low levels per gram dust, adjusted for country, age and sex and sequentially group and dichotomized microbial agent levels.

N=391	PR[a] (CI)	PR[b] (CI)
Model 1		
Medium mite allergen levels (1,390 – 10,424 ng/g)	1.63 (0.89-2.97)	3.13 (1.71-5.73)
High mite allergen levels (> 10,424 ng/g)	0.67 (0.33-1.36)	1.36 (0.67-2.77)
Model 2		
Medium mite allergen levels	1.49 (0.81-2.75)	3.03 (1.66-5.55)
High mite allergen levels	0.67 (0.33-1.37)	1.39 (0.68-2.81)
Group (farm children vs. reference children)	0.66 (0.40-1.07)	0.68 (0.42-1.10)
Group (Steiner children vs. reference children)	0.77 (0.44-1.36)	0.78 (0.45-1.38)
Model 3		
Medium mite allergen levels	1.62 (0.88-2.98)	2.87 (1.51-5.44)
High mite allergen levels	0.77 (0.37-1.57)	1.35 (0.65-2.81)
Group (farm children vs. reference children)	0.87 (0.48-1.56)	0.97 (0.55-1.71)
Group (Steiner children vs. reference children)	0.86 (0.48-1.56)	0.92 (0.51-1.64)
EPS level (above vs. below 84,333 EPS units/g)	0.65 (0.39-1.08)	0.64 (0.38-1.07)
Endotoxin level (above vs. below 10,668 EU/g)	0.82 (0.52-1.30)	0.89 (0.57-1.38)
Glucan level (above vs. below 2,295 ng/g)	1.04 (0.63-1.72)	0.69 (0.42-1.15)

a. Adjusted for country, age and sex
b. Adjusted for age and sex

The correlations between dichotomized microbial agent levels were low (Spearman r < 0.20). Correlations between categorized mite and microbial agents levels (r < 0.23) and between group and dichotomized microbial agents levels were also low (r < 0.40) allowing us to assess their independent effects in multivariate regression analysis.

The shape of the smoothed dose-response curves was essentially the same for below and above median endotoxin and glucan levels in mattress dust (fig. 2), with somewhat lower curves for above median microbial agent levels. For above median EPS levels, however, no indication of a dose-response association between mite allergen levels and mite sensitization was observed.

Table 3 shows the results of regression analyses, with and without adjustment for country. Because country was a determinant of mite allergen levels, the estimated effect of intermediate mite allergen exposure was considerably lower after adjustment for country. Adjusting for group and microbial agent levels did not significantly alter the prevalence ratios for allergen levels. Farm children had a borderline significant lower prevalence of mite sensitization when compared to reference children, after adjustment for mite allergen levels, and the results were similar when a further adjustment for country was made. However, the protective effect of living on a farm largely disappeared after including microbial agent levels in the models. For Steiner children no significant lower prevalence of mite sensitization was observed.

Above median EPS levels were borderline significantly associated with lower prevalence rates of mite sensitization. The interaction between mite allergen levels and EPS was not statistically significant, although figure 2 suggested effect modification by EPS. In the model without adjustment for country indications of a protective effect of glucans were observed as well. Within farm children, protective effects of EPS (PR 0.47 (0.23-0.97)), glucans (PR 0.42 (0.18-1.00)) and endotoxin (PR (0.76 (0.34-1.73)) were observed. In Steiner and reference children non-significant protective effects were observed for endotoxin (Steiner children: PR 0.55 (0.27-1.10)) and EPS (references: PR 0.50 (0.15-1.63)).

DISCUSSION

Highest mite sensitization rates were observed at intermediate mite allergen levels. This non-linear relation was independent of group, country, common risk factors or microbial agent levels. Only for above median EPS levels, indications of modification of the non-linear relationship were observed. The prevalence of mite sensitization was lower in farm children than in reference children, which appeared associated with levels of EPS and glucans in mattresses.

Our results are not in line with previous cohort studies showing a linear dose-response relationship between mite allergen levels and mite sensitization in children in Germany and the United Kingdom [3, 25]. This might be related to differences in allergen levels or differences in study populations. The reported association in the German cohort study refers to allergen levels in living rooms [3], with a median level of 184 ng/g, which is much lower than mattress levels reported in this paper (median 2,465-5,352 ng/g, depending on group). Living room samples in this study also showed much lower allergen levels (median 221-812 ng/g) when compared to mattress samples and were therefore not further studied. Both the German and English cohort study consisted, in part at least, of children considered at special risk of developing allergy, because of allergy or asthma in their parents. A cohort study conducted in the United States showed that parental allergy or asthma might be an important modifier; for children with a parental history of allergy and asthma, a positive association between mite allergen levels and mite sensitization was observed, whereas the opposite was found for children without a parental history [26]. However, our study showed similar dose-response relations for children with and without parental asthma and/or rhinoconjunctivitis, although the peak of mite sensitization at medium mite allergen levels was higher for children with parental asthma and/or rhinoconjunctivitis. Another cohort study from the United Kingdom, which was conducted in a community sample instead of a selection of high-risk children, did not show a linear relationship between mite allergen levels and mite sensitization. This study showed a rising risk of IgE sensitization at low allergen levels and an attenuated risk at high levels, comparable to our results [27]. A similar pattern was observed for the association between cat allergen levels and cat sensitization [27]. A cross-sectional study showed attenuated risks of cat sensitization at high cat allergen levels [28]. It was suggested that high exposure to cat allergen may produce a form of tolerance characterized by a 'modified' Th2 response with specific IgG4 production [28]. Our data suggest that such a mechanism, if it exists, may also apply to mite allergen levels, depending on mite allergen levels and other possible modifying factors like parental allergy or asthma.

An alternative explanation for the attenuated risk of mite sensitization at high mite allergen levels could be that families with allergic or asthmatic members maintain a relatively low allergen environment. This is, however, not supported by our data; few families reported allergen avoidance, and the observed pattern did not change by excluding these people. Also, the same pattern was observed in children whose parents did not report asthma or rhinoconjunctivitis, although prevalences were somewhat lower. Therefore, it is unlikely that allergen avoidance explains the observed pattern with highest mite sensitization rates at intermediate mite mattress allergen levels.

The effect of intermediate mite allergen exposure was much lower after adjustment for country. In Sweden mite allergen levels were very low and only few children were sensitized to house dust mites. This is in agreement with dose-response

associations observed in previous studies [1-4], and should not be corrected for, although the results without adjustment for country might be confounded by other factors differing between countries. However, results adjusted for other risk factors than age and sex (parental education, maternal smoking during pregnancy, current smoking in the household, older siblings, mother's reported asthma and/or rhinoconjunctivitis and father's reported asthma and/or rhinoconjunctivitis) were similar to results adjusted for age and sex alone (PR 2.77 and 1.29 for medium and high mite allergen levels respectively). When Sweden was excluded, highest sensitization rates were still observed at intermediate mite allergen levels (PR 1.70 and 0.74 for medium and high mite allergen levels respectively). We therefore believe that the results not adjusted for country more accurately present the true association between mite allergen exposure and sensitization.

High Der p 1 and low Der f 1 levels were observed in farm children and the prevalence of mite sensitization was low in farm children when compared to reference children. These observations are more or less in line with previous cross-sectional studies in farming or rural populations. The Allergy and Endotoxin (ALEX) study in farm and farm reference children from Germany, Austria and Switzerland, conducted previous to and independent of the PARSIFAL study, also showed 5 times higher mattress Der p 1 levels in farm children than in reference children. Levels of Der f 1 were somewhat, but not significantly lower in farm children than in farm reference children [10]. Despite the high mite allergen levels in farm children, prevalences of mite (*Dermatophagoides pteronyssinus*) sensitization were similar in farm children (15.4%) and farm reference children (15.1%, Waser, unpublished). Studies in adult farmers showed high mite counts or high Der p 1 and low Der f 1 levels in farm homes [5, 6]. The latter study showed that despite high Der p 1 levels, mite sensitization rates were comparable to the prevalence in the general population [5]. Studies in New Zealand and Finland did not show significant differences in mite sensitization between farm and non-farm children [29, 30]. Low overall mite allergen levels were observed in the Finnish study area [29], and in contrast to our study, the New Zealand study observed higher endotoxin levels in non-farm children than in farm children, which might explain why sensitization rates appeared to be higher in farm children than in farm reference children in that study.

Our data suggest that EPS and glucan levels in mattress dust may explain –at least partially– the protective effect of living on a farm on mite sensitization. Indications of a protective effect of endotoxin were observed as well. Correlation between these microbial agents precludes a firm conclusion on the degree to which specific agents contributed to the observed effects, although the correlation was low. In addition, these agents may be markers of a much broader spectrum of microbial agents.

In conclusion, our data suggest a bell-shaped dose-response relationship between mite allergen exposure and sensitization to house dust mites and support the hypothesis that high levels of microbial agents might protect against sensitization.

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CHAPTER 6

Exposure to microbial components and allergens in population studies: A comparison of two house dust collection methods applied by participants and fieldworkers

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ABSTRACT

Background Exposure to indoor allergens and microbial agents is usually estimated by analyzing house dust samples, collected by fieldworkers. Dust collection by study participants would be a practical and cost-effective alternative in large-scale population studies.
Objective We aimed to compare dust weights and allergen and microbial agent levels in fieldworker- versus participant-collected dust samples.
Methods In homes of 216 children, parents collected dust from mattresses and living room floors with nylon socks and, within the same year, fieldworkers collected dust on filters in sampling nozzles (ALK). In 114 homes, fieldworkers also collected dust with socks. Dust samples were analyzed for levels of cat, dog and house dust mite allergens, bacterial endotoxin, mould ß(1,3)-glucans and fungal extracellular polysaccharides (EPS). The differences were investigated by Bland-Altman and correlation plots.
Results Socks appeared to yield less dust from mattresses at relatively low dust amounts and more dust at high dust amounts than ALK samples. Correlations ranged from 0.47 – 0.64 for microbial agents, 0.64 – 0.75 for house dust mite allergens and 0.84 – 0.87 for pet allergens per gram dust in participant-collected socks and fieldworker-collected ALK samples. Cat allergen levels were 2-fold lower and endotoxin levels 3-fold higher in socks than ALK samples. Relative exposure differences between subgroups of the population were, however, similar for ALK and sock samples.
Conclusion Levels of allergens and microbial agents measured in subject-collected socks and fieldworker-collected ALK samples are moderately to highly correlated, but absolute levels may differ, because of differences in particle-size constitution of the dust.

INTRODUCTION

Epidemiological studies on allergies and asthma typically estimate exposure to allergens by analyzing samples of settled house dust. In this way, relationships between house dust mite allergens and allergy have already been established since the sixties [1-6]. During the last decade it has become more and more common to measure in house dust samples also microbial agents, like bacterial endotoxin, since it has been hypothesized that such agents might protect against allergies and asthma [7]. For example, a study in farm and non-farm children showed an inverse relation between endotoxin levels in house dust and atopic diseases in children [8]. In most studies fieldworker-collected dust samples have been analyzed, but in some, including our own PARSIFAL (Prevention of allergy – Risk factors for sensitization in children related to farming and anthroposophic lifestyle) study [9-11], both

fieldworkers and study participants collected house dust in partially overlapping populations.

Fieldworkers collected dust by vacuuming with an ALK (Horsholm, Denmark) sampling nozzle loaded with a filter, to assess allergens and microbial agents in house dust of the PARSIFAL population [10, 12]. Sampling with ALK devices is one of the most commonly used dust sampling methods, which was for example also applied in the International Study of Asthma and Allergies in Childhood (ISAAC) [13]. Participants of the PARSIFAL study collected dust by vacuuming with nylon sample bags [9] ('socks'; Allied Screen Fabrics, Hornsby, Australia), which could, in contrast to ALK devices, be easily mailed to participants, who after sampling returned the socks with dust. This procedure has important logistical advantages for large-scale population studies, like PARSIFAL: the labour costs are much lower than those associated with home visits by fieldworkers, and therefore, the size of the population with measured exposure data can be increased considerably. However, little is known about the reliability of such dust sampling by participants: does it lead to similar results as dust collection by fieldworkers? And does the sock itself lead to similar results as ALK nozzles with filters?

The latter question has been addressed previously in a study in New Zealand, which compared allergen and endotoxin levels in parallel mattress and living room dust samples from homes of 37 children, taken on the same day and by the same fieldworker, with either socks or ALK devices [14]. Moderate to high correlations were observed between the levels of endotoxin, cat and mite allergens per gram dust in the two types of samples (Pearson's r 0.58 – 0.82).

Another recent study addressed the question whether dust sampling by participants leads to similar results as dust sampling by fieldworkers. Cat and mite (Der p 1) allergen levels in dust samples collected by participants were compared with levels in dust samples collected by fieldworkers [15]. Strong correlations between the allergen levels were observed, but participants collected lighter samples, which might have been due to the use of different vacuum cleaners or the use of different dust collection devices; the Mitest Dust Collector (participants) and a paper filter method (fieldworkers).

In this study, we primarily aimed at comparing the two dust sampling methods applied in the PARSIFAL study: dust sampling with socks by participants and dust sampling with ALK devices by fieldworkers. The comparison involves a wide spectrum of biological agents: mite allergens of *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1), cat (Fel d 1) and dog (Can f 1) allergens, endotoxin and two mould components: ß(1,3)-glucans and extracellular polysaccharides (EPS) from *Aspergillus* and *Penicillium spp*. Additionally, we investigated whether differences between the methods could be attributed to the use of different sampling devices by including collection and analysis of extra sock samples, taken by fieldworkers on the same day as the ALK samples.

METHODS

Study subjects

Design and population characteristics of the PARSIFAL study have been described previously [11]. In short, children from farmers' families, anthroposophic families ('Steiner children') and respective reference families of five European countries; Austria, Germany, the Netherlands, Sweden and Switzerland, were included [11]. Parents of participating children who gave consent to dust sampling, with a maximum of 1,000 per country, were sent nylon sampling socks (n=4,892) to collect dust with. Nearly 75% (n=3,625) of sock sampling sets with dust were returned. The contents of these house dust samples were analysed in a case-control selection of nearly 1,000 children, to assess the association between exposure to microbial agents and wheeze, as described previously [9]. In addition, fieldworkers collected house dust with ALK sampling devices in a randomly selected population, stratified by subgroup (farm, Steiner and reference children) to assess levels of microbial agents in each subgroup and to validate the sock method, as described previously [10]. In short, mattress and living room dust was collected in homes of 478 children: 229 farm children, 122 Steiner children and 60 and 67 of their respective references.

Parent-collected socks from 73 homes, which had been analysed within PARSIFAL subprojects other than the previously described case-control study [9], were also included in the comparisons with ALK samples. About half of these additional samples (from 30 homes) were only analysed for dust weights, endotoxin and EPS levels. Over all, both sock and ALK results were available for 216 homes. In more than 75% of these homes, socks had been collected first, and time between the ALK sampling and sock sampling by parents ranged from –128 to 251 days, with a median of 103 days.

To allow a comparison of sampling devices used by the same person on the same day, fieldworkers collected in 114 homes house dust with socks along with ALK sampling. These homes were selected on the basis of floor covering: only homes with wall-to-wall carpets were included in this additional sampling procedure, because low dust weights, as collected on smooth floors, could make comparisons of side-by-side sampling less reliable.

Dust collection

Fieldworkers collected dust with vacuum cleaners equipped with ALK (Horsholm, Denmark) sampling nozzles (fig. 1), loaded with glass fibre filters, as described previously [10], and with nylon sample socks with 25 µm pore size (Allied Screen Fabrics, Hornsby, Australia) (fig. 2). The sample sock was placed into the suction pipe, the upper part of the sock was folded around the outside of the suction pipe and attached with adhesive tape.

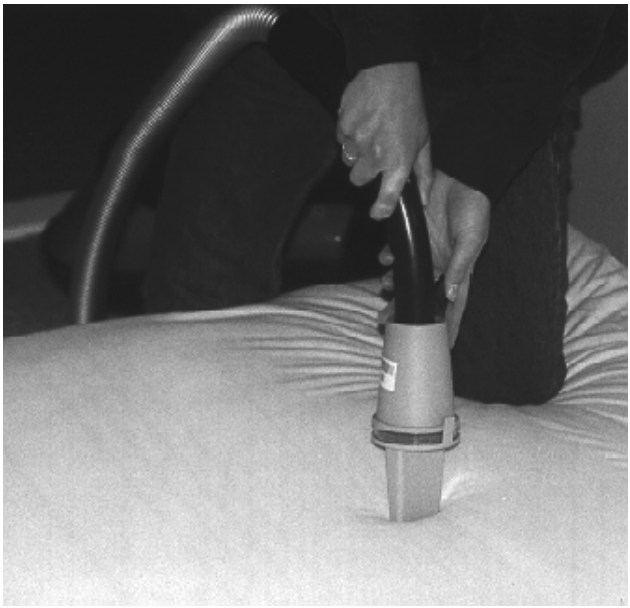


Figure 1. ALK sampling nozzle.

Samples from mattresses were obtained by vacuuming the whole area of the mattress for 2 minutes twice, with both devices, with the order effect controlled for by alternating the first used device. Duplicate living room samples were obtained by vacuuming two adjacent 1 m^2 sites on the carpet for 2 minutes each, with different devices. Other types of floor covering were sampled with ALK devices only: smooth floors with ≥ 4 m² rug, 1 m² of rug, 2 min; smooth floors with no rug or smaller rug(s), 2 m² of smooth floor, 4 min. Filters with dust were transferred to pre-weighed tubes and stored frozen at the various centers, and shipped on dry ice to one laboratory (IRAS, Utrecht, NL) for central analysis.

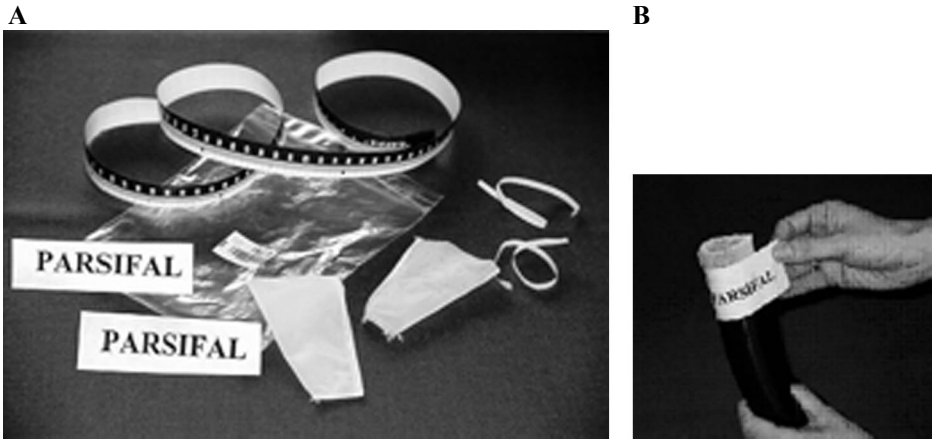


Figure 2. Sampling set for collection of house dust, including two sample socks, a measuring rod, 2 stickers and twist ties (A) and attachment of a sock to the vacuum cleaner with a sticker (B).

Parents collected dust with nylon sample socks, applying the same sock sampling procedures as fieldworkers, which were illustrated in detailed photo-instructions. They were instructed to vacuum the whole area of the mattress with one sock and 1 m^2 of the living room floor with the other sock, for 2 minutes each. Socks with sampled dust were returned to the local study centers by regular mail, where they were stored frozen. The samples were shipped on dry ice to Utrecht.

Dust analysis

The tubes with ALK filters plus dust were post-weighed and 5-40 ml extraction fluid was added, determined by the net dust weight (<0.5 g, 5 ml; 0.5 to 1.0 g, 10 ml; 1.0 to 2.0 g, 20 ml; > 2.0 g, 40 ml). The filter was thus extracted as well. The dust in the sock samples was transferred from the sock to a pre-weighed tube and after post-weighing the tube with dust, 5-40 ml extraction fluid was added according to the same weight-fluid scheme as used for ALK samples. Allergens and microbial agents were extracted from the dust in the same way for socks as for ALK samples, according to a three-step procedure, extracting endotoxin first, followed by EPS and allergen extraction, and finally extraction of glucans, as described previously [10]. Endotoxin was measured with the kinetic chromogenic Limulus Amebocyte Lysate (LAL) test, EPS with a specific sandwich EIA for EPS of *Aspergillus* and *Penicillium spp.* [16] and glucans with an inhibition EIA [17]. Mite allergens of *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1) and the major cat (Fel d 1) and dog (Can f 1) allergens were measured with sandwich EIAs of

Indoor Biotechnologies® as described previously [18]. The average inter-day/inter-assay coefficients of variation, as determined by testing duplicate extract aliquots of 10% of all samples on another day as the first aliquot, were: 30.7% for endotoxin, 13.5% for EPS, 23.4% for glucans, 19.3% for Der p 1, 17.6% for Der f 1, 18.6% for Fel d 1 and 25.9% for Can f 1.

Data analysis

Results were expressed as concentrations per gram of dust and per square meter. For samples with undetectable amounts of dust (< 0.020 g, less than 5% of all samples), no concentrations per gram of dust were calculated. Samples with non-detectable amounts of dust or biological components were not included in the quantitative comparisons between methods regarding that specific, non-detectable component. Percentages of non-detectable values were low for microbial agents (< 4%) and cat allergens (<6%), moderate for dog allergens (11-35%) and high for house dust mite allergens (16-24% of mattresses; 43-48% of floor samples). The majority of samples with non-detectable levels of mite allergens were samples from Sweden, which has known low house dust mite allergen levels. Dust collection methods were also evaluated by comparing the sum of Der p 1 and Der f 1, because this parameter was used in previous analyses in the PARSIFAL population [12]. In quantitative comparisons of the sum of Der p 1 and Der f 1, all samples with a detectable amount of at least one of the two allergens were included.

Statistical analyses were performed with SAS statistical software (version 8.2, SAS Institute, Cary, N.C.). Levels of allergens and microbial agents were approximately log-normally distributed after exclusion of samples with non-detectable levels. Pearson correlation coefficients were calculated for ln-transformed values of dust and biological agent levels. To evaluate the agreement between dust sampling methods and to test whether differences depended on the level of the agents, Bland-Altman plots [19] were produced. In these plots, differences in ln-transformed levels between the methods were plotted against the mean level. Differences in ln-transformed levels were further evaluated by performing t-tests for paired observations and they were also plotted against the time interval between dust collection by parents and fieldworkers. We primarily compared dust and biological agent levels in parent-collected socks with levels in ALK samples taken by fieldworkers. Additionally, levels in ALK samples were compared with levels in socks, collected on the same day by fieldworkers.

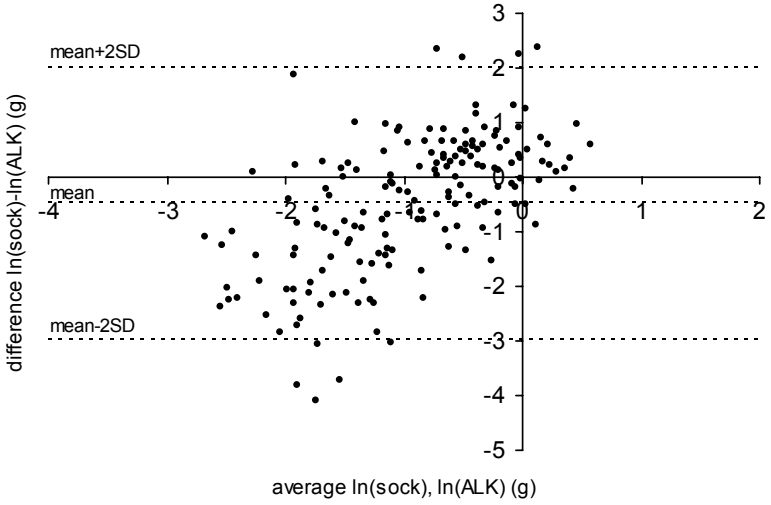
RESULTS

Dust yields

Parent-collected socks versus fieldworker-collected ALK samples
The amounts of mattress dust were on average 1.5 times lower in parent-collected socks than in ALK samples (0.31 vs. 0.47 g, $p<0.01$). The difference was in fact underestimated, because 30 of these ALK samples had been taken directly after sock sampling by the fieldworker; if these 30 samples were excluded, mean ALK mattress dust weights were 0.50 g. For living room floors, mean dust weights were similar in socks collected by parents and ALK samples (0.32 vs. 0.35 g, $p=0.43$). Pearson correlation coefficients for the amounts of dust in the two types of samples were low to moderate: 0.24 for mattresses and 0.43 for living room floors. Figure 3 shows that for living rooms, the difference between measurements did not depend on the mean dust weight, whereas it appeared that mattress socks showed 3 to 15 times lower dust weights at low mean dust amounts. In contrast, at high mean mattress dust amounts, sock dust weights were 2-3 times higher.

Fieldworker-collected sock and ALK samples
Amounts of mattress dust in fieldworker-collected sock samples were 1.7 times lower ($p<0.01$) than in ALK samples, collected by the same fieldworker on the same day, whereas living room floor dust amounts were not significantly different. The order in which the two mattress samples had been taken determined the dust yield; dust amounts in socks were 1.2 times lower when the sock method was applied to the mattress first, but 2.1 times lower when the ALK device was applied first. The Bland-Altman plot for mattress dust weights of fieldworker-collected socks and ALK samples showed the same trend as figure 3 for parent-collected socks and ALK samples, with relatively low sock dust weights at low mean dust levels (not shown). Pearson correlation coefficients between fieldworker-collected sock and ALK dust levels were, on the other hand, higher than those between parent-collected socks and ALK samples: r was 0.70 for mattress dust and 0.79 for living room floor dust.

A



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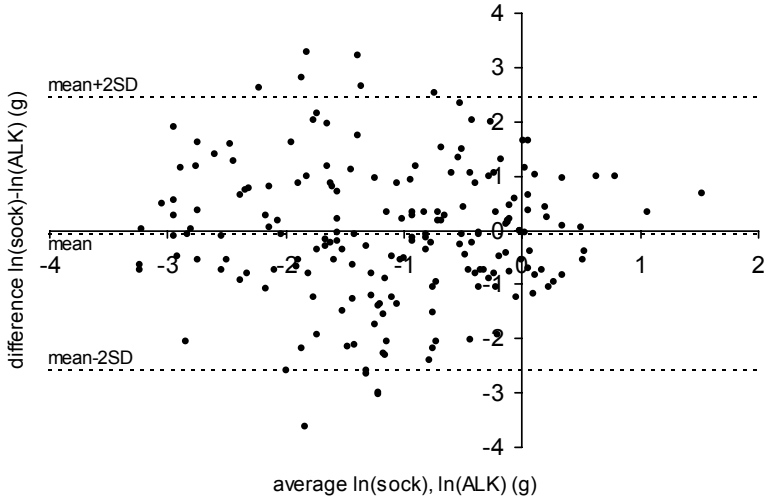


Figure 3. Bland-Altman plots showing differences between ln-transformed dust weights in parent-collected socks and fieldworker-collected ALK samples[a], for A) the mattress B) the living room (SD=standard deviation).
a. 30 mattress ALK samples taken on the same day, shortly after sampling with socks by fieldworkers, were excluded.

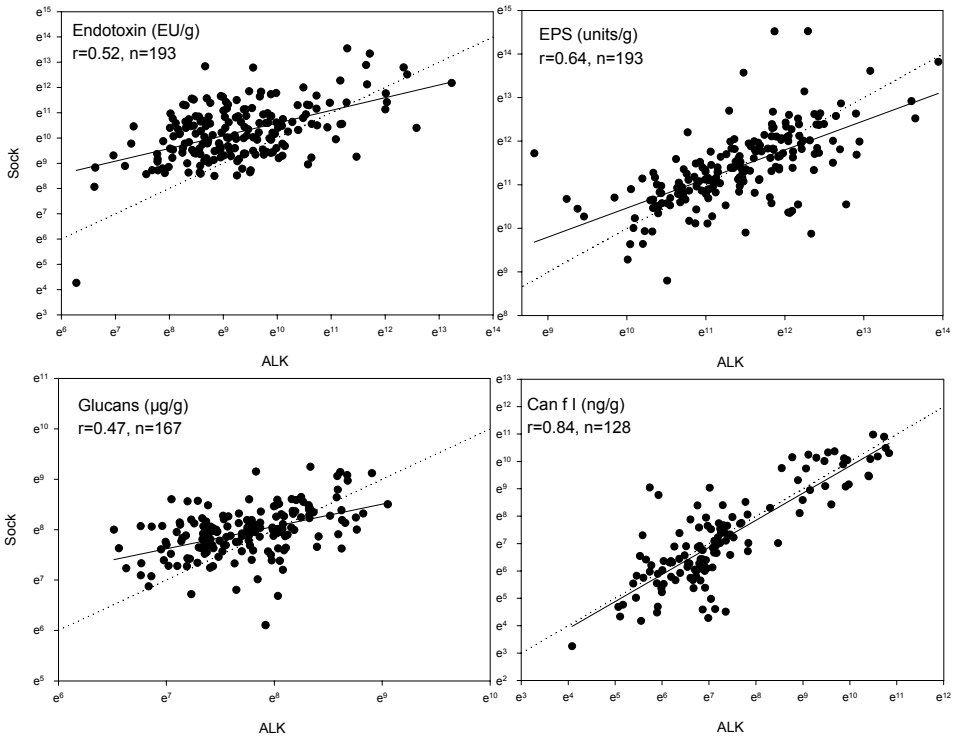


Figure 4. Correlations between mattress levels of biological agents per gram dust in ALK samples and parent-collected socks, with Pearson correlation coefficients (r), regression line (solid line) and line of unity (dotted line).

Biological agent levels

Parent-collected socks versus fieldworker-collected ALK samples

Figure 4 shows correlations between levels of biological agents per gram of mattress dust measured in ALK samples and parent-collected socks. Levels of endotoxin, EPS and glucans per gram dust were moderately correlated between the sampling methods (Pearsons' r ranging from 0.47 to 0.64). Correlations were high for allergen levels (r 0.64 - 0.87). Generally, correlations were higher with wider ranges in levels of the specific component; the 10% and 90% percentile values of Fel d 1, for example, differed more than 500 orders of magnitude and Fel d 1 showed a high correlation coefficient, whereas for glucans this difference was only 4-fold, and the correlation was moderate.

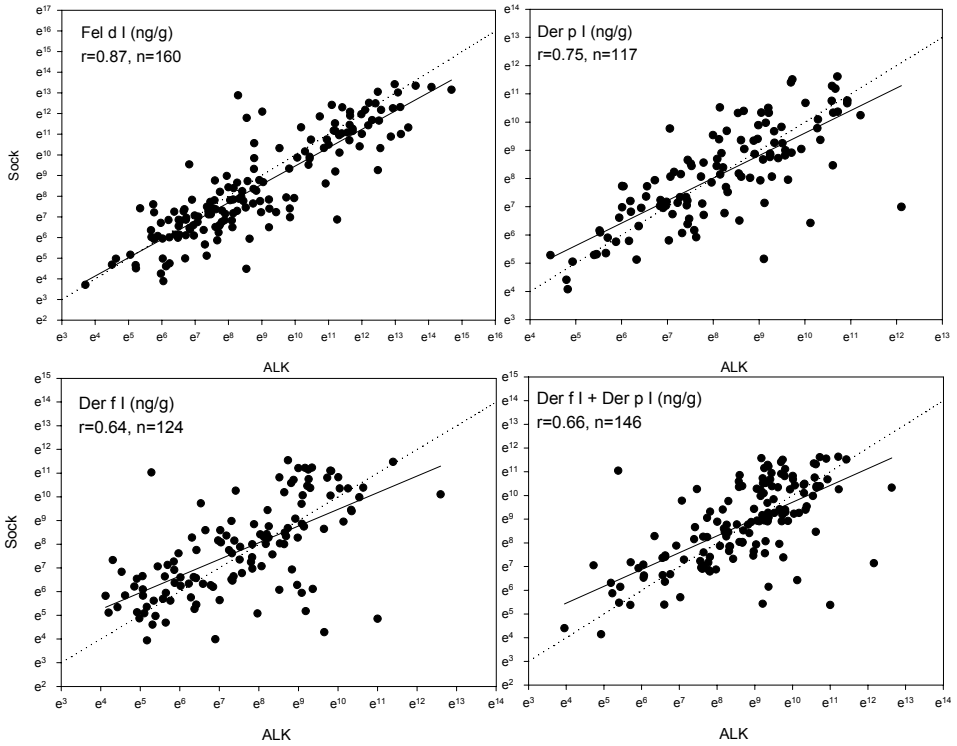


Figure 4 – continued

Figure 5 shows the correlations for living room floor samples, which were generally somewhat lower than for mattresses, but with the same overall pattern for the various agents. When allergen and microbial agent levels were expressed per square meter of sampled area (table 1), correlation coefficients for floor samples were very similar to those found per gram dust, whereas for mattress samples, the correlation coefficients for levels per square meter were consistently lower, in accordance with the above mentioned lower correlation between dust yields from mattresses, compared to those from living room floors.

Correlations between biological agent levels were similar when levels below the limit of detection, given a value of two-third of the lowest detectable amount, were included in the analyses (not shown). For example, for Can f 1, Der p 1 and Der f 1, which could not be detected in relatively many samples, correlation coefficients for levels per gram mattress dust were 0.69, 0.84 and 0.76 after inclusion of non-detectable samples,

respectively. The concordance (percentage detectable or non-detectable on both occasions) for these agents ranged from 66.3 to 82.0%.

Bland-Altman plots in which differences in biological agent levels were plotted against the time interval between sock sampling by parents and ALK sampling by fieldworkers suggested that the time interval played no major role (shown in fig. 6 for endotoxin and Der p 1). This was confirmed by restricting correlation calculations to ALK and sock samples taken more than the median number of days apart, which resulted in similar values as those found for the total dataset.

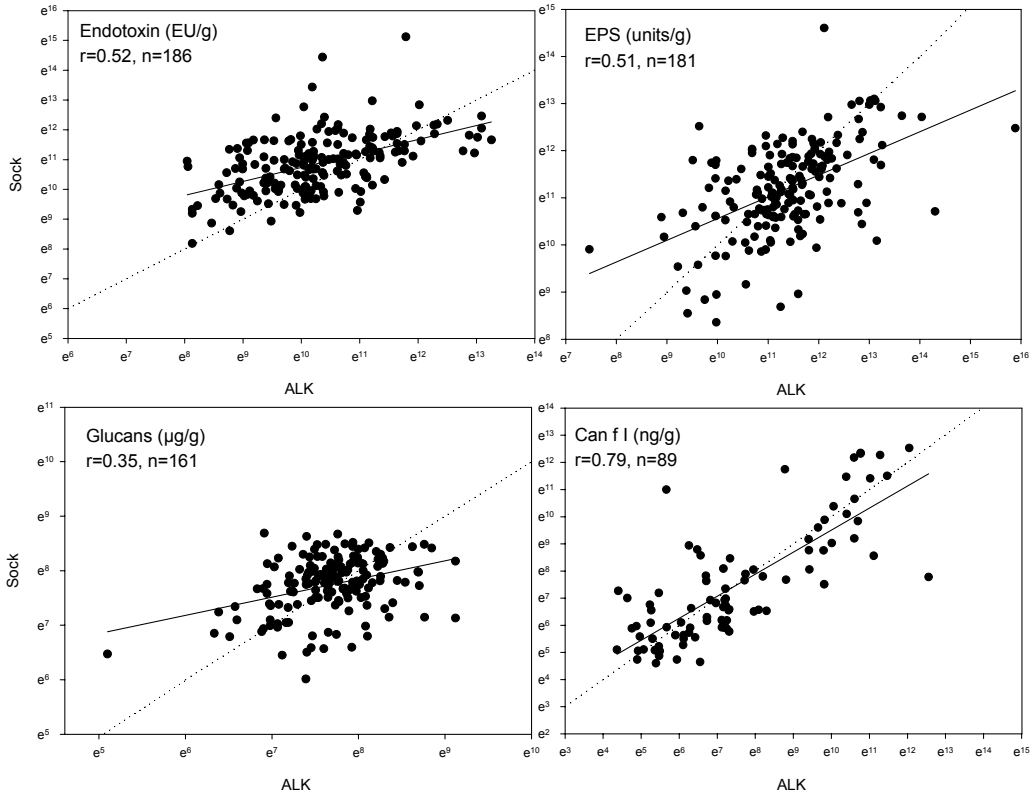


Figure 5. Correlations between living room floor levels of biological agents per gram dust in ALK samples and parent-collected socks, with Pearson correlation coefficients (r), regression line (solid line) and line of unity (dotted line).

The correlation plots in figure 4 and 5 show that in general (>80% of measurements), levels did not differ more than a factor 7.4 between the methods. Bland-Altman plots showed that the mean difference in ln-transformed levels between methods was not significantly ($p<0.05$) different from 0 for most components in mattress and living room dust (not shown). Exceptions were cat allergen levels, which were 1.5-2.0 times lower, both in mattress and living room floor sock samples, and endotoxin levels, which were 1.8-2.6 times higher in sock than in ALK samples. Bland-Altman plots showed that mean differences were not dependent on the mean concentrations (not shown).

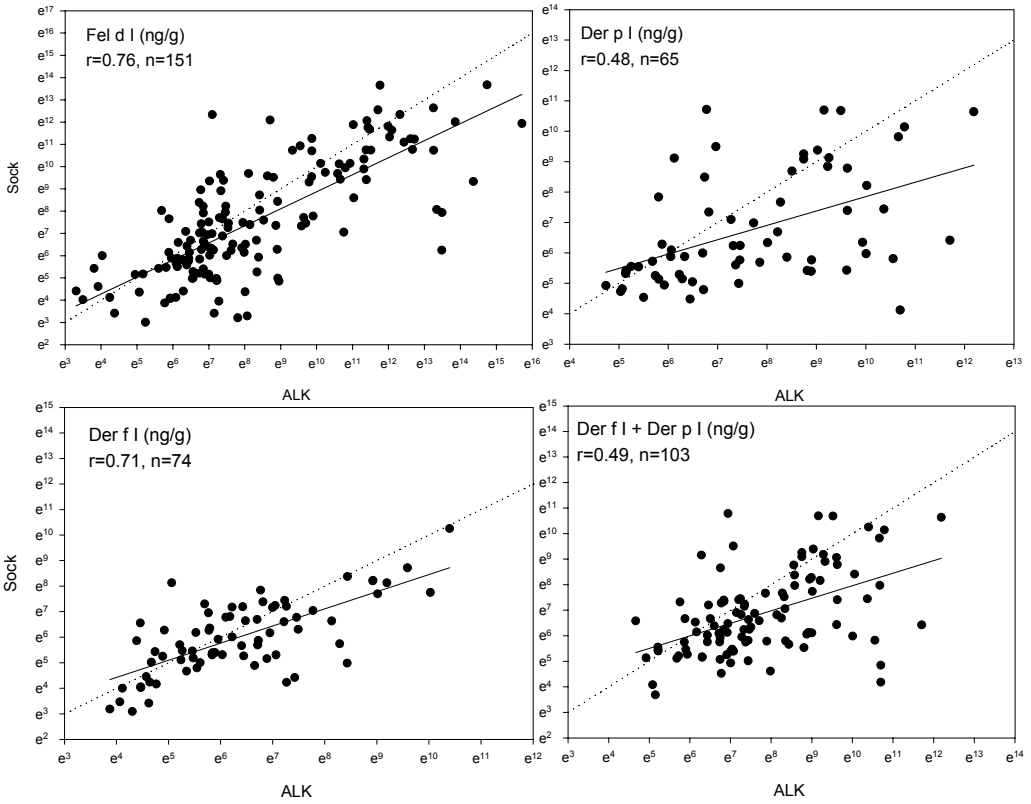


Figure 5 – continued

Table 1. Pearson correlation between levels of biological agents in ALK samples and parent-collected socks, expressed per square meter sampled mattress and living room area.

	Mattress		Living room	
	Ndet[a]	r	Ndet[a]	r
Endo, EU/m^2	201	0.43	203	0.55
EPS, units/m^2	201	0.43	197	0.47
Gluc, µg/m^2	174	0.34	174	0.51
Can f 1, ng/m^2	133	0.70	98	0.70
Fel d 1, ng/m^2	168	0.74	161	0.71
Der f 1, ng/m^2	127	0.60	78	0.64
Der p 1, ng/m^2	124	0.67	65	0.52
Der p 1 + Der f 1, ng/m^2	152	0.61	107	0.58

a. Number of sample pairs with detectable levels of the specific agent in both sock and ALK samples.

Fieldworker-collected sock and ALK samples

Correlations between biological agent levels in ALK samples and fieldworker-collected socks (table 2) were generally higher than correlations between levels in ALK samples and parent-collected socks (figs. 4, 5). Especially for mite allergens there was a striking difference: Der p 1 and Der f 1 levels in fieldworker-collected sock and ALK samples showed much higher correlations (r values 0.79 to 0.93) than the comparisons of levels of these agents in fieldworker-collected ALK and parent-collected sock samples.

Differences in mean levels of cat allergen and endotoxin were also observed between ALK samples and fieldworker-collected socks. Cat allergen levels were 1.7-1.9 times lower ($p<0.01$) in fieldworker-collected sock mattress and living room samples, and endotoxin levels were 1.2-1.3 times higher ($p<0.05$ for mattresses and $p<0.10$ for living rooms) in fieldworker-collected sock samples than in ALK samples.

Table 2. Pearson correlation between levels of biological agents per gram dust in ALK samples and fieldworker-collected socks.

	Mattress		Living room	
	Ndet[a]	r	Ndet[a]	r
Endo, EU/g	98	0.66	104	0.57
EPS, EPS units/g	99	0.58	106	0.67
Gluc, µg/g	99	0.59	105	0.27
Can f 1, ng/g	74	0.84	76	0.87
Fel d 1, ng/g	96	0.93	101	0.92
Der f 1, ng/g	79	0.91	68	0.79
Der p 1, ng/g	71	0.93	56	0.89
Der p 1 + Der f 1, ng/g	88	0.92	84	0.85

a. Number of sample pairs with detectable levels of dust and the specific agent in both sock and ALK samples.

Thus, for endotoxin, differences in levels between fieldworker-collected socks and ALK samples were smaller than those between parent-collected socks and ALK samples, which suggests that, next to sampling device, fieldworker- versus participant dust collection, or the day of dust sampling, influenced mean levels of endotoxin. No effect of sampling order was seen on levels of biological components per gram mattress dust (not shown).

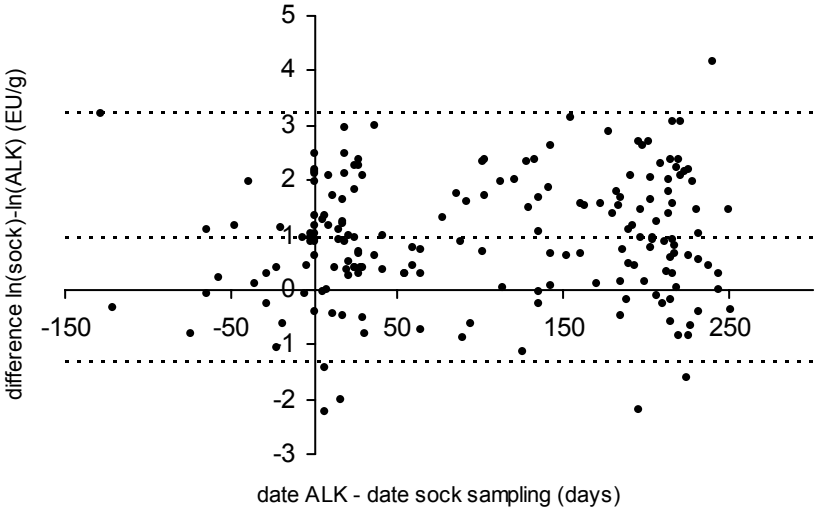
Microbial agents across subgroups of the PARSIFAL population, using ALK samples or parent-collected socks

Table 3 validates parent-collected dust samples, by comparing previously published results of ALK samples [10] with results of the same analyses using parent-collected socks. All analysed socks were included in these data-analyses (1,284 children), not only socks which matched with ALK samples, to increase power. Geometric means and standard deviations of microbial agent levels in mattress and living room dust were calculated for farm children, Steiner children, farm reference children and Steiner reference children. The data-analyses with microbial agent levels in parent-collected socks lead to similar conclusions as in the published report [10]. Because of larger numbers of sock samples, differences between subgroups, e.g. Steiner children and their references, more often reached statistical significance (table 3). Also for pet and mite allergens, relative exposure differences across subgroups were very similar for ALK and sock data (not shown).

Table 3. Validation of previously published results [10]; levels of microbial agents in the subgroups of the PARSIFAL population, as determined by ALK sampling, compared to levels determined by sock sampling by parents. *, $p < 0.05$; **, $p < 0.01$ for levels in farm and Steiner children versus levels in their respective references.

Microbial agent	Method	Farm		Farm-ref		Steiner		Steiner-ref	
		N	GM (GSD)	N	GM (GSD)	N	GM (GSD)	N	GM (GSD)
Mattress									
Endotoxin (EU/g)	ALK	227	16,548 (3.0)**	59	6,322 (2.8)	121	9,687 (2.9)	64	7,422 (3.2)
	Socks	382	33,835 (3.1)**	258	17,940 (3.0)	321	23,515 (2.6)**	178	17,316 (2.8)
EPS (units/g)	ALK	223	118,247 (2.1)**	58	48,271 (2.2)	121	69,575 (2.4)*	67	50,395 (2.4)
	Socks	381	118,294 (2.8)**	259	50,507 (3.1)	323	61,359 (2.8)**	182	40,790 (3.5)
Glucans (μg/g)	ALK	224	2,440 (1.7)**	58	1,928 (1.8)	121	2,396 (1.7)*	67	1,937 (2.0)
	Socks	271	3,276 (1.8)**	190	2,359 (2.0)	326	3,040 (2.0)**	145	2,144 (2.6)
Living room									
Endotoxin (EU/g)	ALK	221	44,035 (3.1)**	56	13,874 (5.0)	114	21,608 (3.0)	63	17,871 (4.1)
	Socks	370	62,883 (2.7)**	240	35,517 (2.9)	336	42,741 (2.8)	169	38,289 (3.0)
EPS (units/g)	ALK	219	123,186 (3.2)**	54	40,098 (3.7)	112	56,890 (2.4)*	63	34,838 (4.8)
	Socks	376	99,742 (3.4)**	245	39,769 (4.1)	337	52,369 (2.8)*	172	39,999 (4.0)
Glucans (μg/g)	ALK	222	2,083 (2.0)	53	1,732 (2.5)	114	2,269 (1.8)	60	2,010 (2.2)
	Socks	271	2,541 (2.2)**	184	1,864 (2.1)	338	2,435 (1.9)	133	2,236 (2.0)

A



B

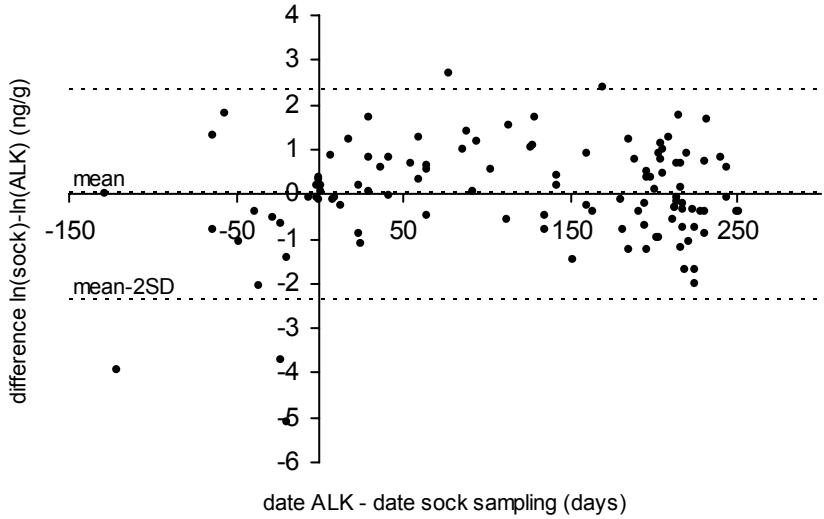


Figure 6. Bland-Altman plots showing differences between ln-transformed biological agent levels in parent-collected socks and fieldworker-collected ALK samples against the time interval between them, for 2 examples; A) endotoxin per gram mattress dust B) Der p 1 per gram mattress dust (SD=standard deviation).

DISCUSSION

Our data showed that dust collection by participants is a reliable and practical option for allergen and microbial agent exposure assessment, because of high return rates for subject-collected socks (nearly 75%) and moderate to high correlations with levels of biological components in fieldworker-collected dust. This paper confirmed previous findings of allergen levels in participant- and fieldworker-collected dust samples [15], whilst adding information of microbial agent levels. We also investigated the differences that were observed between the methods, i.e. in dust weights and cat allergen and endotoxin levels per gram dust, which may not be important for large epidemiological studies into relative exposure differences across groups, but which might be of importance for other studies. Our data do not provide a direct answer to the question to what extent differences in levels between parent-collected socks and ALK samples could be attributed to either the time interval or the person who performed the sampling (fieldworker versus parent). However, we included comparisons with socks collected by fieldworkers to investigate the effect of using different devices.

Person who performs the sampling: fieldworkers versus participants
Although dust yields from socks were in the same order of magnitude as dust amounts from ALK samples, correlations between the two methods were only moderate for dust weights, which is in line with a previous report of participant- and fieldworker-collected dust samples [15]. Differences between yields of fieldworker- versus participant-collected dust samples might be due to the use of different kinds of vacuum cleaners or differences in dust sampling procedures; despite our detailed photo-instructions, it might have been more difficult for parents than for trained fieldworkers to strictly follow the vacuuming instructions (i.e. vacuum one square meter of the living room floor for exactly 2 minutes). However, the low correlations between subject- and fieldworker collected dust amounts could, in our study, also be due the time interval or the device.

Time interval
The time interval between sock sampling by parents and sock/ALK sampling by fieldworkers ranged from –128 to 251 days. One would expect to find larger random differences and thus lower correlations at larger time intervals. We observed higher correlation coefficients for dust yields and mite allergen levels, when dust was collected by fieldworkers on the same day with either ALK or sock devices, which suggests that time interval indeed influenced mean levels, although we could not detect effects in comparing samples taken several days or months apart. Our correlations between levels in parent-collected socks and ALK samples were generally higher than correlations between allergen levels in ALK samples taken 2 years apart (Antens, IRAS, Utrecht, personal

communication), which also suggests that time interval between measurements influences correlations.

Different devices

Logically, correlations in this study could not be higher than correlations between levels in duplicate samples, collected on the same day with the same device by the same person. A study comparing such duplicate samples, collected with ALK devices, showed similar correlations as our comparisons of fieldworker-collected socks and ALK samples (Giovannangelo, IRAS, Utrecht, personal communication), suggesting that application of sock sampling by fieldworkers leads to similar results as ALK sampling by fieldworkers. This has previously been concluded from a study comparing ALK and sock samples collected by fieldworkers, which assessed endotoxin, mite and cat allergen levels, and which showed similar correlations as our study [14]. However, there appeared to be some differences in dust yields and composition of the dust between ALK samples and sock samples, no matter whether socks were taken by fieldworkers or parents. Mattress dust amounts appeared to be lower in socks, particularly at low dust levels. ALK sampling of mattresses was easier because the sampling nozzle could be kept at a fixed angle, while with sock sampling the sheets on the mattresses sometimes stuck to the vacuum cleaner pipe and thereby reduced contact with the mattress itself. In addition, dust may have been lost when transferring dust from socks to tubes before extraction, although no differences in dust amounts were observed for living room socks as compared to ALK samples. Interestingly, however, figure 3 showed that the mattress dust yield did not only depend on collection method, but also on the mean dust weight, with relatively low sock dust weights at low mean dust levels and relatively high sock dust weights at high mean dust levels. Differences in pore sizes (socks 25 μm, filter < 1 μm) might explain this discrepancy. Filters might be more efficient at low dust weights, with large amounts of fine dust, whereas socks might be more efficient in capturing larger particles. The differences were not observed for living room floor dust, suggesting that these differences depend on the constitution of dust.

Cat allergen levels were up to 2-fold higher in ALK samples than in socks, and endotoxin levels appeared to be up to 3-fold higher in parent-collected socks than in ALK samples. Cat allergen is the predominant allergen among particles < 2.5 μm [20], which might have been captured by glass fibre filters (pores < 1 μm) more easily than by socks (pores 25 μm). Differences in particle size distribution of sock or ALK dust might have influenced endotoxin levels as well. However, as epidemiological studies usually investigate relative exposure differences between subgroups, e.g. people with and without a particular disease, absolute levels are generally less relevant. We showed that, despite differences in absolute levels (i.e. for endotoxin), comparisons of relative microbial exposure differences between groups showed similar results for ALK samples and socks, collected by parents.

Conclusions

Dust collection with socks by parents appears to be as valid for allergen and microbial agent exposure assessment as dust collection with ALK sampling devices by fieldworkers. Dust yields, and maybe even dust particle-size distributions may differ between methods, therefore, absolute levels of biological agents are not (always) comparable between studies using different dust collection methods, even when expressed per gram dust.

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CHAPTER 7

Discussion

Research described in this thesis was part of the PARSIFAL study, that aimed 1) to investigate whether differences in atopic disease prevalences between farm children or children from anthroposophic families and respective references, as observed previously [1-5], could be confirmed in a large population of children from 5 European countries and 2) to explore factors which might explain the lower prevalence of atopic diseases in these children. The work in this thesis specifically focused on the role of allergens and microbial agents in house dust. In this chapter, the results will be critically evaluated against the current literature on this topic, including PARSIFAL papers focusing on other potentially protective factors than allergens and microbial agents, like diet. More specifically, the following items will be discussed: validity of exposure and health outcome measurements in this thesis, and evidence for protective factors of growing up on a farm or in a family with an anthroposophic lifestyle.

Validity of measurements of allergens and microbial agents in house dust

House dust components were measured in two types of samples: samples collected by fieldworkers with ALK devices (results presented in Chapter 3 and 5) and samples collected by parents with socks (Chapter 4). Chapter 6 described comparisons of biological agent levels in these two types of samples. Allergen levels were highly correlated between the two types of samples, whereas microbial agent levels were moderately correlated. The results of Chapter 3, showing higher microbial agent levels in farm children, and to a lesser extent, in Steiner children, as compared to their references, were confirmed by calculations with levels in socks instead of levels in ALK samples. This does not only suggest that dust collection with socks by study participants is as reliable as dust collection by fieldworkers, but also that, within the context of large populations studies, a single measurement at one point in time is sufficiently precise to estimate exposure to microbial components over fairly extended periods, as socks and ALK samples were taken up to 251 days apart. However, it is unknown to what extent single exposure measurements reflect long-term exposure, i.e. from birth till the age of inclusion in our study (at 5 to 13 years). Only few studies have included repeated measurements of allergens and/or microbial agents in house dust over a time period of several years. Topp et al. observed low to moderate correlations between pet and mite allergen and endotoxin levels in dust samples taken up to 6 years apart and it was concluded that a single measurement does not accurately reflect long-term exposure [6]. Recent data from a follow-up study of Dutch children also suggested that single measurements of Der p 1 and Der f 1 are not sufficient to estimate long-term exposure, but it was suggested that one single measurement of Fel d 1 actually might be sufficient (Antens, IRAS, Utrecht, personal communication). Notably, cat allergen levels in our study were comparable to levels in the Dutch study, but house dust mite allergen and endotoxin levels in our study were much higher than in these previous studies, therefore, their results might not be directly applicable to our study.

We estimated exposure to microbial agents by measuring microbial agent levels in house dust. It is unknown however, whether this is a useful proxy of 'true' exposure to microbial agents. Assuming that inhalation is the most relevant route of exposure for airway diseases, one could argue that airborne levels could be better proxies of exposure to these agents. Few studies have measured airborne and house dust associated endotoxin levels in the home environment, and these studies showed a low correlation between levels in the dust and in airborne samples, suggesting that levels in dust are no surrogate for airborne levels [7-9]. Some studies assessed indoor airborne glucan levels in relation to airway diseases, as reviewed by Rylander [10], but the correlation with levels in dust was not assessed so far. For house dust mite allergens, only moderate correlations between airborne levels and levels in settled dust were observed, even when dust was artificially resuspended into the air, e.g. by vacuuming [11-14]. One would preferably estimate long-term exposure to allergens and microbial agents based on a series of repeated airborne measurements. However, measurement of airborne biological agents requires very sensitive assays, because of low amounts of collected dust, and the commonly applied methods lack this sensitivity. Secondly, airborne levels are highly variable in time, therefore, they might be very relevant when studying short-time health effects, but this variability makes it difficult to estimate long-term exposure, which is probably more relevant for health endpoints measured in the PARSIFAL study, such as atopy. Thus, surface dust may reflect long-term exposure to airborne biological components better than a single airborne measurement.

We measured biological agents in mattress dust, living room floor dust and settled stable dust. Dust from stables contained up to 7-fold higher levels of microbial agents than living room floor samples of farm children, with living room floor levels in farm children being up to 3-fold higher than those in farm reference children. Assuming that the airborne dust concentrations in stables are approximately 20 times higher than in living rooms, with up to 7*3 times higher microbial agent levels per gram dust, then exposure in stables could be up to 420 times higher than indoors and spending 10 minutes in a stable would be equivalent to spending 3 days indoors of homes of farm reference children. Thus, the difference in microbial exposure between farm children and their rural references is not just up to 3-fold, as assessed by their difference in indoor levels, but between 3- and 420-fold, though closer to 3 than 420, because time spent in stables is probably short compared to time spent indoors. It would be interesting to estimate exposure of farm children by personal air sampling, e.g. by carrying pumps sampling air close to the mouth, as often applied in occupational settings [15-17], because not only levels in stables, but also activities and time spent in stables will influence exposure. Some previous studies have used nasal air samples to estimate personal exposure to pet, mite or occupational allergens or endotoxin [18-22], which might also be useful to estimate exposure in farm children. Exposure measurements by personal air sampling would, however, impose a large effort, and would therefore not be possible in all participants of a large population study like

PARSIFAL. However, extensive measurements in a subgroup of children could provide the basis for a model to estimate exposure of all children, both indoor and in stables, by exposure determinants, such as type of stable, time spent in stables, etcetera. Such models have previously been used in occupational studies, such as studies in adult farmers [15, 16, 23].

Validity of health outcomes

Main health outcomes used in this thesis were 'atopic wheeze' and 'house dust mite sensitization'. The parameter 'wheeze', as assessed by questionnaire was validated within the framework of the PARSIFAL study, by comparing this outcome with bronchial hyperresponsiveness (BHR) to hypertonic saline, in a population of 319 children with wheeze and 459 children without wheeze [24]. BHR is a useful diagnostic tool in assessing asthma, but should not be considered the gold standard, because not all children with asthma show hyperresponsiveness in this test and vice versa [25-28]. The association between BHR and reported wheeze was similar for the four subgroups of the PARSIFAL population; farm children, farm reference children, Steiner children and Steiner reference children, which suggests that the four subgroups showed a similar understanding of the health outcome questions on wheeze, despite potential differences in individual perception as well as consulting habits across these subgroups [24]. Therefore, it seems unlikely that differences in atopic wheeze prevalences, as presented in Chapter 4, were influenced by different understandings of the questions on wheeze across subgroups. Interpretation issues neither could influence sensitization to house dust mites, as this was assessed by IgE measurements in blood samples.

It could not be determined whether the relations between microbial agents and atopic wheeze in farm and farm reference children, as described in Chapter 4, reflected a down-regulation of the development of atopy and/or the development of wheeze. Because atopic non-wheezers were not included in the case-control selection, the effects of microbial agents on atopy, independent of wheeze, could not be studied. It would have been interesting to study these effects to see whether they would be smaller, larger or similar to the effects we observed on atopic wheeze, because it is not known whether microbial agents protect from the development of atopy alone, or also from the development of asthma in atopic and/or non-atopic children. The previously published cross-sectional ALEX (Allergy and Endotoxin) study showed a negative association between endotoxin levels and atopy and atopic wheeze, but for non-atopic wheeze, the association was in the other direction, suggesting that endotoxin mainly protects against atopy [29]. Endotoxin might even be a cause of non-atopic asthma [30]. However, the questions regarding the influence of microbial agents on the development of atopy and wheeze should preferably be addressed in a birth-cohort study.

One could speculate about the effects of living on a farm on atopy and/or wheeze by calculation of the fraction of wheezing or asthma cases that is attributable to atopy. If atopy has an odds ratio for asthma of R, then the proportion of atopic cases that are attributable to atopy is (R-1)/R, and the proportion of all cases in the population that are attributable to atopy (population attributable risk) is P(R-1)/R where P is the proportion of all cases that are atopic [31]. In our population, the proportion of current wheeze cases that could be attributed to atopy was 60%. For asthma this population attributable fraction was 49%, which is in line with estimates from several other cross-sectional studies, which ranged from 25-63%, as shown by Pearce et al. [31], despite the artificial composition of our study population. The proportions of wheeze and asthma cases which could be attributed to house dust mite sensitization were 43% and 32% respectively, in our population. Chapter 2 showed that living on a farm has an odds ratio of 0.53 (0.42-0.67) for atopic sensitization. Thus, farm children are at about half the risk of developing atopy, and thereby, without an additional effect on the development of wheeze, the wheezing prevalence would be reduced by 0.5*0.6[a]=0.3*100=30%. As shown in Chapter 2, the odds ratio for wheeze is 0.78 (0.62-0.99), which is in line with this 30% reduction, which suggests that living on a farm merely protects against atopy rather than wheeze.

Protective factors of an anthroposophic lifestyle

Chapter 2 of this thesis showed a lower prevalence of atopy and atopic diseases in children from Steiner schools compared to reference children, but the differences could not be confirmed in each country. Microbial agents in house dust did not explain the lower prevalence of atopic wheeze in children from Steiner schools. It is not likely that microbial agents in house dust explain the lower prevalence of atopy or other allergic diseases in these children either, because differences in microbial agent levels between Steiner children and their references were small. House dust mite allergen levels were similar in Steiner children and their references (table 1, Chapter 5). Dog allergen levels neither differed between these groups of children (not shown), but cat allergen levels were somewhat (1.6 times) higher in homes of Steiner children compared to reference children. As allergen levels were not or only marginally different between Steiner children and their references, it is not likely that allergen levels explain the lower prevalence of atopy or other allergic diseases in Steiner children.

a. Also when using the population attributable risk for the combined group of farm and farm reference children (0.5 instead of 0.6), the reduction in wheeze prevalence (25%) is in line with the OR for wheeze.

In contrast to microbial agents and allergens in dust, lifestyle factors strongly differed between Steiner children and their references [32]. Use of antibiotics and/or antipyretics, especially during the first year of life, was associated with increased risks of hay fever, asthma and eczema and MMR vaccination was associated with an increased risk of hay fever [32]. Thus, other routes or sources of exposure to immunomodulating agents appear to protect Steiner children from developing allergic diseases than inhalation of microbial agents and allergens. For instance, the gut microflora, which was shown to be different between children of parents with or without an anthroposophic lifestyle [33], perhaps due to differences in antibiotics use and diet, might play an important role in the observed differences. However, the lower prevalence of atopic diseases in children from anthroposophic families can probably not be explained by one single factor and also factors which we did not (adequately) measure might play a role, as suggested by two parents from Steiner children (appendix 1).

Protective factors of growing up on a farm

Farm children were exposed to similar house dust mite (Der 1) allergen levels, when compared to the other groups of children. Cat allergen levels were 3.9-fold higher (table 1, Chapter 5) and dog allergen levels were 2.2-fold higher (not shown) in farm children than in farm reference children. Thus, the lower prevalence of atopic diseases in farm children cannot be attributed to lower allergen levels. Only Der f 1 levels were lower in farm children than in farm reference children, but in contrast, Der p 1 levels were higher in farm children than in their references. Little data are available on the allergenic potency of Der p 1 versus Der f 1, but recent data from a Dutch birth cohort study showed that Der p 1 and Der f 1 were equally related to sensitization to house dust mites [34]. Some studies have suggested that pets might protect against sensitization and/or asthma [35-37], but other studies showed no or positive associations between pets and atopy or asthma [37-40]. Interestingly, despite low numbers of cat-sensitized children, we could confirm findings of Platts-Mills et al. [41], showing attenuated risks of cat sensitization at high cat allergen levels (not shown). However, in view of conflicting literature, it seems unlikely that high pet allergen levels explain the lower prevalence of atopic diseases in farm children.

Another explanation for the lower prevalence of atopic diseases in farm children could be the ‘healthy farmer effect’, i.e. a selective avoidance of farming by atopic families. Chapter 2 showed lower prevalences of atopic diseases in farm children, also when results were adjusted for atopic diseases in parents of the participating children, but the selection effects could have taken place generations before. However, only 5% of the parents of farm children and 2.5% of rural reference children indicated that their ancestors quitted farming because of allergies or asthma. The ‘healthy farmer effect’ will further be addressed in a separate publication, by comparing genetic background of the children.

A recent study on PARSIFAL farm and farm reference children showed that the consumption of farm milk was negatively associated with hay fever, wheeze and asthma [42]. Drinking farm milk could be protective because farm milk, usually consumed raw, contains more gram-negative bacteria than pasteurized milk [4]. Thus, the effect of farm milk consumption could be attributed to exposure to microbial products through another route of exposure: ingestion rather than inhalation. Alternatively, the higher fat content of farm milk could explain this finding: previous studies showed a negative association between full cream milk consumption and asthma [43, 44].

The data presented in chapter 4 and 5 of this thesis suggest that microbial agents in house dust protect against atopic diseases, but the effect of living on a farm was still largely unexplained by indoor microbial agent levels. The parameter 'living on a farm' is less subject to measurement error when compared to measurement of microbial agent levels, which might explain why it was a stronger determinant. In addition, the difference in microbial exposure between farm children and their references is probably underestimated by taking only house dust levels, and not levels in stables, into account.

Stable visits or participation in haying protected against hay fever, and stable visits of the mother during pregnancy protected against sensitization in the child [42]. This is in line with the hypothesis that microbial agents protect against atopic diseases, because previous studies [45, 46] and Chapter 3 of this thesis showed high microbial agent levels in stables. Endotoxin, EPS and glucans only represent a part of the heterogeneous mixture of agricultural dusts, which not only contain agents of plant, animal or microbial origin, but also animal dander and urinary poteins and ammonia. This mix of agents might also have adjuvant effects, as shown for glucans and endotoxin [47, 48]. It is also possible that microbial agents modify the immune response to allergens as shown in murine models [49-51] and experiments with peripheral blood monocytes [52]. The latter hypothesis was addressed in Chapter 5, which showed that the shape of dose-response associations between house dust mite allergens and sensitization was similar for above and below median microbial agent levels, but the overall prevalence of sensitization was lower at high microbial agent levels. For EPS, indications of modification of the dose-response curve were observed. The effects of high exposures to all of three microbial agents were further investigated by summing these (normalized) levels, but results were similar to the effects of single agents (not shown). Further studies are needed to investigate contents and immunomodulatory effects of stable dust.

Conclusions

The lower prevalences of atopic diseases in farm children compared to rural reference children, as observed previously, were confirmed in the 5 countries of the PARSIFAL study. The lower prevalences of atopic diseases in children of Steiner schools compared to their references, as previously observed in Sweden, could also be confirmed, but not in

every country. Microbial agents, nor pet and mite allergens in house dust could explain the lower prevalence of atopic diseases in Steiner children. Endotoxin, glucans and EPS in house dust appeared to be negatively associated with atopy and atopic wheeze in farm children and these agents may partially account for the protective effect of living on a farm. House dust mite allergen levels were similar in farm and farm reference children and in view of conflicting literature on the effects of pets on atopy and asthma, it seems unlikely that higher pet allergen levels, as observed in farm compared to reference children, could explain the lower prevalences of atopic diseases in this group.

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APPENDIX 1

Textbox 1. Letter from a PARSIFAL mother, in which she gives her vision on the question why farm children and children from families with an anthroposophic lifestyle have less allergies and asthma. She explains that asthma is caused by a lack of an open environment for children, to develop their selves physically, emotionally and spiritually. Farm children and children of Steiner schools grow up in a more open environment: farm children live in extended areas, and children of Steiner schools get much time to play, as Steiner schools do not only nurture the intellectual, but also the psychological and spiritual unfolding of the child. This open environment could protect these children from developing asthma.

Jullie schrijven dat het opvallend is dat kinderen met een agrarische achtergrond en kinderen uit families met een antroposofische leefstijl minder vaak allergie dan wel astma hebben. Vandaar jullie onderzoek. Ik vind het heel logisch dat kinderen van boerderijen en uit antroposofische families minder last hebben van astma. Astma is een aandoening die op geestelijk of zielsniveau te maken heeft met ruimte(beknotting). De ruimtebeknotting is de oorzaak van astma, het stof is het middel wat dan als katalysator werkt. Kinderen op boerderijen hebben vaak heel veel ruimte om zich heen. Ook kinderen uit antroposofische gezinnen krijgen veel speelruimte, vaak niet letterlijk, maar wel figuurlijk, in de zin van 3 kleuterschooljaren, waarin echt gespeeld mag worden. Op de lagere school komt het intellectuele leren pas langzaam op gang. Het blijft bijzonder speels, veel speelruimte dus. Dat is mijns inziens de oorzaak dat astma minder vaak voorkomt bij de 'boerderij / antroposofische' kinderen. Ik wilde jullie mijn visie niet onthouden.

Een PARSIFAL moeder.

Textbox 2. A PARSIFAL mother asks herself whether she has an anthroposophic lifestyle, upon our question in the questionnaire. Does she need to take into account her bathing frequency, the way she dresses or her frequency of watching television? Fragments from a letter in a Steiner school paper.

Een antroposofische leefstijl, heb ik die?

Uit de vragenlijst van het onderzoek naar oorzaken van astma en allergie bij kinderen, maak ik op dat de onderzoekers ervan uitgaan dat ik snap wat zij bedoelen met een antroposofische leefstijl, maar wat zij daarmee voor ogen hebben is me niet duidelijk. Toen ik nog op school zat, was me wel duidelijk wat een 'soof' was. Ze waren meestal zwaar op de hand: bogen van de mondhoeken richting aarde en wenkbrauwen richting hemel. Vrouwelijke exemplaren herkende je aan knotjes, lange rokken en wijde verhullende jasjes, het liefst in lila, de mannelijke met name aan het dragen van sandalen in de winter. Wanneer de visuele kenmerken niet duidelijk genoeg waren, kon je altijd nog ruiken of er een echte 'soof' in de buurt was. De echten gingen namelijk niet graag in bad. Dit kenmerk hebben de wetenschappers mogelijk in hun definitie betrokken, gezien de passage in de begeleidende brief over 'blootstelling aan bepaalde stoffen' die het immuunsysteem mogelijk activeren. Trouwens, de vele zuurkoolvragen in de vragenlijst doen vermoeden dat de wetenschappers ook een verhoogde zuurgraad op het spoor zijn.
De vraag naar mijn levensbeschouwing vind ik op zich wel mooi. Daarmee geven de onderzoekers aan dat ze ervan uitgaan dat de gedachten die wij, ouders en opvoeders, erop na houden van invloed zijn op de gezondheid van onze kinderen. Dat vind ik een wijze en verstrekkende gedachte. Ik probeer daar rekening mee te houden, alleen lukt het me in de praktijk lang niet altijd daarnaar te leven.
Moet ik de vraag met 'ja' of 'nee' beantwoorden? Als ik teruggrijp op het overzichtelijke lijstje waarmee we ons op school behielpen lijkt het antwoord simpel. Mijn zuurgraad kan ik niet objectief inschatten, maar wijde jasjes heb ik niet, lange rokken draag ik 'minder dan 1 tot 6 keer per week', knotten lukt zelden en waarschijnlijk ga ik ook nog te vaak in bad. Daarnaast hebben we geen Moeder aarde van sprookjeswol op de Jaartafel. We hebben wel weer lappenpoppen, maar zijn die specifiek antroposofisch? Onze kinderen spelen gewoon en doen geen computerspelletjes. De tv hebben ze wel eens aan gezien, maar ze vinden het nog steeds niet raar dat hij meestal uit staat. Is dit volgens de onderzoekers kenmerk van de antroposofische leefstijl (overigens geen gekke gedachte, ikzelf ben heel allergisch voor de ononderbroken herrie van auto's, snelwegen en andere toeters en bellen)? Waarom vragen de onderzoekers dan niet gewoon hoe vaak je in bad gaat of hoe vaak de televisie aanstaat?
Kortom, ik ben er niet uitgekomen. Ik heb mijn antwoord, door het aankruisen van meerdere hokjes tegelijk, geprobeerd zo meerduidig te maken als de vraag. Ik hoop maar voor de onderzoekers dat mijn huisstof een duidelijker antwoord oplevert....Misschien is dat wel ondubbelzinnig antroposofisch!

SUMMARY

Over the past decades, the prevalence of allergic diseases in childhood has increased considerably, especially in many western industrialized countries. The rising prevalence might be due to changes in allergen exposure, early infections and/or lifestyle factors. The PARSIFAL (Prevention of Allergy - Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) project focused on two groups of children who have shown a relatively low prevalence of atopic diseases and sensitization: farm children and children of families with an anthroposophic lifestyle. Contact with farm animals has been associated with a decrease in the risk of atopic disease, which might be due to exposure to microbial compounds. The anthroposophic way of life involves several characteristics, such as restrictive use of antibiotics, antipyretics and vaccinations, as well as certain dietary habits, which might be negatively associated with atopic diseases.

Main objectives of the PARSIFAL study were 1) to investigate whether differences in atopic disease prevalences between farm children or children from anthroposophic families and respective references, as observed previously, could be confirmed in a large population of children from 5 European countries and 2) to explore factors which might explain the lower prevalence of atopic diseases in these children. The work in this thesis specifically focused on the role of allergens and microbial agents in house dust.

Chapter 2 showed that prevalences of atopic diseases were lower among farm children than among farm reference children in all 5 participating countries: Austria, Germany, Sweden, Switzerland and The Netherlands. Also children from Steiner schools, whose parents predominantly adhere to an anthroposopic lifestyle, showed lower prevalences of atopic diseases, but the differences with reference children were less pronounced and not as consistent between countries.

Chapter 3 showed that farm children were exposed to 1.2- to 3.2-fold higher levels of endotoxin, ß(1,3)-glucans and fungal extracellular polysaccharides (EPS) in house dust. Dust from stables contained up to 21-fold higher levels of microbial agents than living room floor samples of farm reference children. Steiner school children were exposed to 1.1- to 1.6-fold higher levels of these agents.

Chapter 4 focused on the association between microbial agent levels in house dust and atopic wheeze. Mattress microbial agent levels were not associated with atopic wheeze in Steiner children and their references, whereas they appeared to protect against atopic wheeze in farm children and their references. Not only bacterial endotoxin, but also mould components might offer some protection against atopic wheeze. However, the effect of living on a farm was still largely unexplained by microbial agent levels, which might be due to more measurement error in microbial agent levels than in the parameter ‘living on a farm’ and because levels in stables were not taken into account.

Chapter 5 focused on allergen levels in house dust. House dust mite and dog allergen levels were similar in Steiner children and their references, but cat allergen levels were somewhat (1.6-fold) higher in homes of Steiner children compared to reference children. Cat allergen levels were 3.9-fold higher and dog allergen levels were 2.2-fold higher in farm children than in farm reference children. Farm children were exposed to similar house dust mite allergen levels, when compared to the other groups of children, but they showed lower house dust mite sensitization rates. A bell-shaped dose-response relationship between mite allergen exposure and sensitization to house dust mites was observed. The shape of the curve was similar for children who were exposed to relatively high or relatively low microbial agent levels, but the overall prevalence of sensitization was lower for children exposed to high levels than those exposed to low levels, which supports the hypothesis that high levels of microbial agents might protect against sensitization.

Chapter 6 showed that levels of pet and mite allergens and microbial agents were moderately to highly correlated between the two house dust collection methods applied in the PARSIFAL study, i.e. sampling with ALK sampling nozzles by fieldworkers (results presented in Chapter 3 and 5) and sampling with nylon socks by parents (Chapter 4). The results of Chapter 3, based on microbial agent levels in ALK samples, were confirmed by results based on microbial agent levels in sock samples. Therefore, it was concluded that dust collection by participants is a reliable and practical option for allergen and microbial agent exposure assessment.

Taking together the results of this thesis, it can be concluded that microbial agent levels in house dust may partly account for the protective effect of living on a farm to atopy and/or wheeze. The protective effect of an anthroposophic lifestyle, as observed in some countries but not in others, could not be explained by microbial agent levels. The relatively low prevalence of atopic diseases in these groups of children could not be attributed to lower pet or mite allergen levels. Higher pet allergen levels, as observed in the homes of these children, could be related to the lower prevalence of atopic diseases, but this seems unlikely in view of conflicting literature on this topic.

SAMENVATTING

De laatste 30 tot 40 jaar is het vóórkomen van allergische aandoeningen bij kinderen aanzienlijk gestegen, met name in Westerse landen. Deze stijging kan mogelijk worden toegeschreven aan veranderingen in leefstijl of in blootstelling aan allergenen of infecties op jonge leeftijd. Opvallend is dat boerenkinderen en kinderen van ouders met een antroposofische leefstijl minder kans op allergieën en astma lijken te hebben dan leeftijdsgenoten. Eerdere studies hebben laten zien dat contact met landbouwhuisdieren mogelijk geassocieerd is met een lager risico op allergische aandoeningen, door daarmee samenhangende blootstelling aan micro-organismen. De antroposofische leefstijl heeft verschillende kenmerken, zoals een lager gebruik van antibiotica en pijnstillers, minder vaccinaties en andere voedingsgewoonten, die mogelijk samenhangen met een lagere kans op allergische aandoeningen.

De PARSIFAL (Prevention of Allergy - Risk factors for Sensitization In children related to Farming and Anthroposophic Lifestyle) studie had ten doel om:

1. De prevalentie van allergische aandoeningen te meten bij boerenkinderen, kinderen uit families met een antroposofische leefstijl en controlekinderen uit 5 Europese landen, om te kijken of eerder aangetoonde verschillen in prevalenties in deze populatie bevestigd konden worden. De controlegroep voor boerenkinderen bestond uit kinderen die op het platteland, maar niet op een boerderij woonden. De controlegroep voor Vrije School kinderen, die vaak ouders met een antroposofische leefstijl hebben, bestond uit kinderen die in dezelfde omgeving woonden, maar niet op een Vrije School zaten.
2. Om factoren te onderzoeken die deze verschillen mogelijk verklaren. De studies in dit proefschrift hebben betrekking op de rol van allergenen en microbiële agentia in huisstof.

Allergische aandoeningen bleken in alle 5 landen minder vaak voor te komen bij boerenkinderen dan bij controlekinderen. Ook kinderen van Vrije Scholen hadden gemiddeld een lagere prevalentie van allergische aandoeningen, maar de verschillen met hun controlekinderen waren minder uitgesproken dan bij de boerenkinderen en werden ook niet in alle landen gevonden (hoofdstuk 2).

Boerenkinderen waren blootgesteld aan hogere niveaus van bacterie- (endotoxine) en schimmelcomponenten (glucanen en extracellulaire polysaccharides (EPS)): concentraties hiervan waren 1,2- tot 3,2-maal hoger in huisstof van boerderijen dan bij controles en in stallen waren de concentraties 21-maal hoger dan in huisstof van de controlekinderen. Bij Vrije School kinderen werden 1,1- tot 1,6-maal hogere concentraties van deze componenten gevonden dan in huisstof van controlekinderen, maar deze verschillen waren niet of nauwelijks significant (hoofdstuk 3).

Hoofdstuk 4 beschrijft de relatie tussen microbiële componenten in huisstof en het voorkomen van atopie in combinatie met astmaklachten (piepen op de borst) bij kinderen. Er werd geen verband gevonden tussen microbiële componenten in matrasstof en atopie en astmaklachten bij Vrije School kinderen en hun controlekinderen, maar onder boerenkinderen en hun controles waren deze componenten geassocieerd met een lagere kans op atopie en astmaklachten. Niet alleen bacterieel endotoxine, maar ook schimmelcomponenten hebben mogelijk een beschermend effect op atopie en astmaklachten. Het beschermende effect van het wonen op een boerderij kon echter maar voor een klein deel verklaard worden door microbiële componenten in huisstof.

Hoofdstuk 5 beschrijft allergeenconcentraties in huisstof en de relatie met een atopische immuunrespons tegen huisstofmijtallergenen (kinderen die zo'n respons hebben zijn 'gesensibiliseerd'). Concentraties van huisstofmijt- en hondallergenen waren vergelijkbaar tussen Vrije school kinderen en hun controles, maar niveaus van katallergenen waren 1,6-maal hoger bij Vrije School kinderen. Bij boerenkinderen werden 3,9-maal hogere kat- en 2,2-maal hogere hondallergeenconcentraties gevonden dan in huisstof van controlekinderen. Hoewel boerenkinderen blootgesteld waren aan vergelijkbare concentraties huisstofmijtallergenen in huisstof, waren ze toch minder vaak gesensibiliseerd tegen deze allergenen dan de controlekinderen. Onderzoek van de dosis-respons relatie tussen huisstofmijtallergenen per gram stof en de prevalentie van huisstofmijt-sensibilisering liet zien dat in alle groepen de hoogste prevalentie werd gevonden bij een gemiddelde blootstelling. De vorm van de dosis-respons relatie was hetzelfde voor kinderen die aan hoge of lage concentraties van microbiële componenten waren blootgesteld, maar de prevalentie van sensibilisering was lager voor hoogblootgestelde kinderen. Deze bevinding komt overeen met de hypothese dat microbiële componenten in huisstof mogelijk bescherming bieden tegen de ontwikkeling van een atopische immuunrespons.

Hoofdstuk 6 gaat nader in op de twee methoden die zijn toegepast voor het verzamelen van huisstof in het kader van de PARSIFAL studie, namelijk stofzuigen met ALK monsternamekoppen door veldwerkers (resultaten hiervan staan in hoofdstuk 3 en 5) en stofzuigen met nylon zakjes door ouders van de deelnemende kinderen (hoofdstuk 4). De concentraties van allergenen en microbiële componenten in stof correleerden redelijk tot goed tussen beide methoden. De verschillen in blootstelling tussen kinderen, beschreven in hoofdstuk 3 op basis van concentraties in stof uit ALK-monsters, werden bevestigd met de resultaten op basis van concentraties in door ouders verzameld stof. De conclusie van hoofdstuk 6 was dan ook dat het laten verzamelen en opsturen van huisstof door deelnemers aan het onderzoek een betrouwbaar en praktisch alternatief is voor het verzamelen van huisstof door veldwerkers.

Op basis van de resultaten in dit proefschrift kan geconcludeerd worden dat de hogere concentraties microbiële componenten in huisstof de lagere prevalenties van atopie en astmatische klachten bij boerenkinderen ten dele kunnen verklaren. Deze componenten konden de lagere prevalenties van allergische aandoeningen bij Vrije School kinderen niet verklaren. De concentraties van huisdierallergenen waren wat hoger bij boerenkinderen en Vrije School kinderen dan bij controlekinderen. Toch is het niet waarschijnlijk dat dat de oorzaak is van de lagere kans op atopische aandoeningen, gezien de tegenstrijdige bevindingen in de literatuur op dit gebied.

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CURRICULUM VITAE

Dieneke Schram-Bijkerk was born on February 23rd, 1975 in Haarlemmermeer, the Netherlands. In 1993 she graduated from secondary school at the Ichthus College in Enschede and started with the MSc program of Environmental Sciences at Wageningen University. In 1998 she graduated in Occupational and Environmental Health and subsequently started working as an epidemiologist at the National Institute of Public Health and the Environment (RIVM) in Bilthoven. She worked at the Centres for Public Health Forecasting (1998-1999) and Environmental Health Research (1999-2000) of RIVM. In 2000, she started working on the study described in this thesis, at the Institute for Risk Assessment Sciences (IRAS) of Utrecht University. Additionally, she worked on the International Study of Asthma and Allergy in Childhood (ISAAC).

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