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Allergy is a worldwide problem and the most common chronic disease among children. Its prevalence is generally low in non-affluent countries but has increased alarmingly over the past decades in affluent countries. On the other hand, soil-transmitted helminth (STH) infections -which mostly affect children- are endemic in non-affluent countries while these infections are uncommon in affluent countries. These opposing trends led to the idea that the two phenomena may be inversely associated. This association has been the focus of much epidemiological research in the last decade(s). So far, most research has been cross-sectional of design and longitudinal research is still scarce. This thesis will focus more on this longitudinal association between STHs and allergy in children.

Allergy: atopic diseases and allergic sensitization

Atopic diseases are inflammatory disorders characterized by hypersensitivity of the immune system to harmless environmental allergens. Atopic diseases include asthma, allergic rhinoconjunctivitis, and atopic dermatitis (1-4).

Asthma is a disorder characterized by chronic inflammation of the airways. During asthmatic episodes the airways are reversibly obstructed. Asthma can occur at any age, but most commonly arises during (late) childhood. Asthma in childhood can remit and recur, but asthma in adulthood mostly persists. No standard definition exists for asthma. Asthma is in general diagnosed based on clinical symptoms, e.g. wheeze, dyspnoea, cough, and objective measures, e.g. variable airflow obstruction (1, 4, 5).

Allergic rhinoconjunctivitis (AR) or hay fever is a disorder characterized by inflammation of the nasal mucosa. Also AR can present itself at any age, but most commonly arises during childhood. No objective measure for AR is available and the diagnosis is made based on symptoms, e.g. nasal congestion, rhinorrhoea, pruritus, and sneezing, which are easily and often incorrectly attributed to viral upper respiratory tract infections (2, 4, 5).

Atopic dermatitis (AD) or eczema is a disorder characterized by inflammation of the epidermis. AD mostly arises before the age of five. AD is diagnosed based on the presence of significant pruritus, which mostly worsens during the evening and night, and eczematous lesions with a typical shape and distribution (3-5).

The development of atopic diseases is characterized by a typical sequence which is often referred to as the “atopic march”. AD is usually the first atopic disease to manifest itself with its onset in early infancy, often followed by asthma and then by AR (6, 7). Approximately half of the children with AD will progress to asthma and two third will progress to AR (6).

Allergic sensitization or atopy is “a personal or familial tendency to produce IgE antibodies in response to low doses of allergens, usually proteins, and to develop typical symptoms such as asthma, rhinoconjunctivitis or dermatitis” (8). While allergic sensitization and atopic diseases often go hand in hand, sometimes children are atopic without manifesting atopic diseases or children have atopic diseases without experiencing allergic sensitization (7). In this thesis, allergic sensitization and atopic diseases will be collectively called allergy.

Worldwide allergy is very common with asthma being the most common chronic disorder in children (9-11). Asthma affects 300 million people and is expected to affect 100 million more by 2025 (5, 9). The prevalence of asthma worldwide is shown in Figure 1. Allergy mostly causes morbidity and no mortality except for asthma. Globally asthma causes 386,000 annual deaths which is 0.7% of all deaths or 14 in every 1000 deaths (12). Allergy poses a significant burden on the individual patient, family, healthcare services and society (5, 9, 10, 13-17) and bring about enormous economic costs (5, 13, 15, 16, 18). Per year 25 million disability-adjusted life years (DALYs) are lost by asthma -0.9% of all DALYs lost worldwide- which made it the 29th leading cause of DALYs lost in 2012 and this is comparable with disorders like protein-energy malnutrition, anxiety disorders and hypertensive heart disease (12).

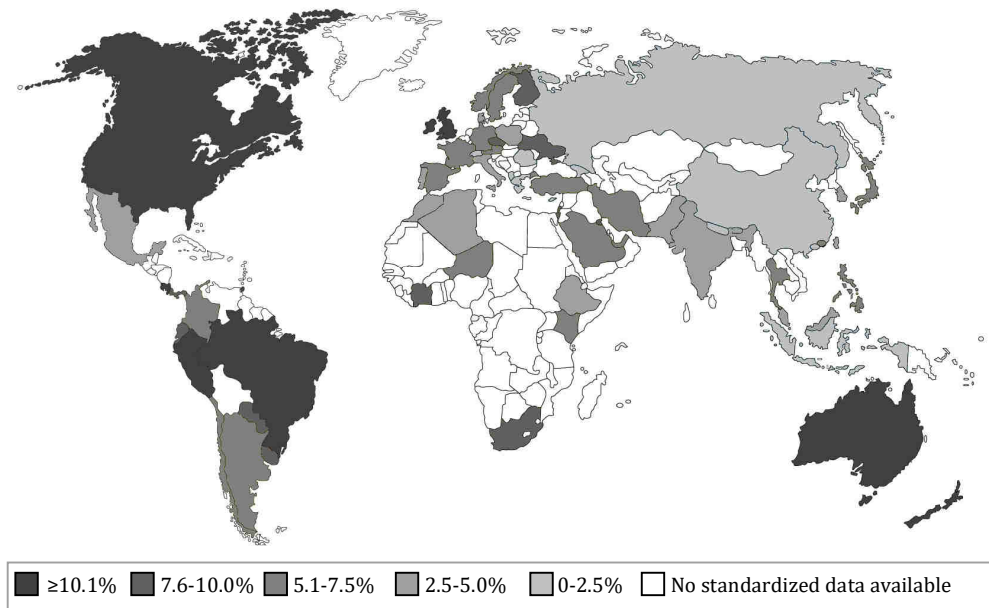


Figure 1. Worldwide prevalence of clinical asthma (Reproduced with permission from (9)).

Allergy is in particular prevalent in urbanized and affluent populations and the prevalence has increased alarmingly over the past decades (19-23). Allergy has a multifactorial aetiology which include genetic, environmental and host factors (24, 25). However, the rapid increase in allergy, lower prevalence in less affluent populations compared to affluent populations, and higher prevalence in urban than rural populations in the same country point towards a major role of environmental factors in the development of allergy (1, 25, 26).

The increase in allergy led to the foundation of a large worldwide epidemiological research programme, the International Study of Asthma and Allergies in Childhood (ISAAC). The programme's goal is to globally assess the prevalence and their trend over time, the severity, and the aetiology of allergy. For this purpose a range of methods has been defined and developed, which are now also widely applied in epidemiological research on allergy beyond this ISAAC programme (27-30).

Soil-transmitted helminths

Helminth infections are caused by parasitic worms and most commonly result in chronic infections in humans. Helminth infections hardly cause mortality, but morbidity on the other hand is a large problem (31). Soil-transmitted helminths (STHs) or geohelminths are an important group of helminths. This name is related to the fact that their eggs need a developmental period in the soil before they become infective. The most important STHs are *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (*Ancylostoma duodenale* and *Necator americanus*), which respectively infect between 807-1221, 604-795, and 576-740 million children globally (32). STHs together with *Schistosoma spp* are responsible for 40% of the disease burden due to tropical diseases, other than malaria (33). STHs cause malnutrition, anaemia, reduction in iron status, poor growth and delayed cognitive development (32, 33). The estimated disability-adjusted life years (DALY) lost due to STHs ranges from 4.7 million to 39 million worldwide depending on the emphasis put on cognitive and physical health effects (32).

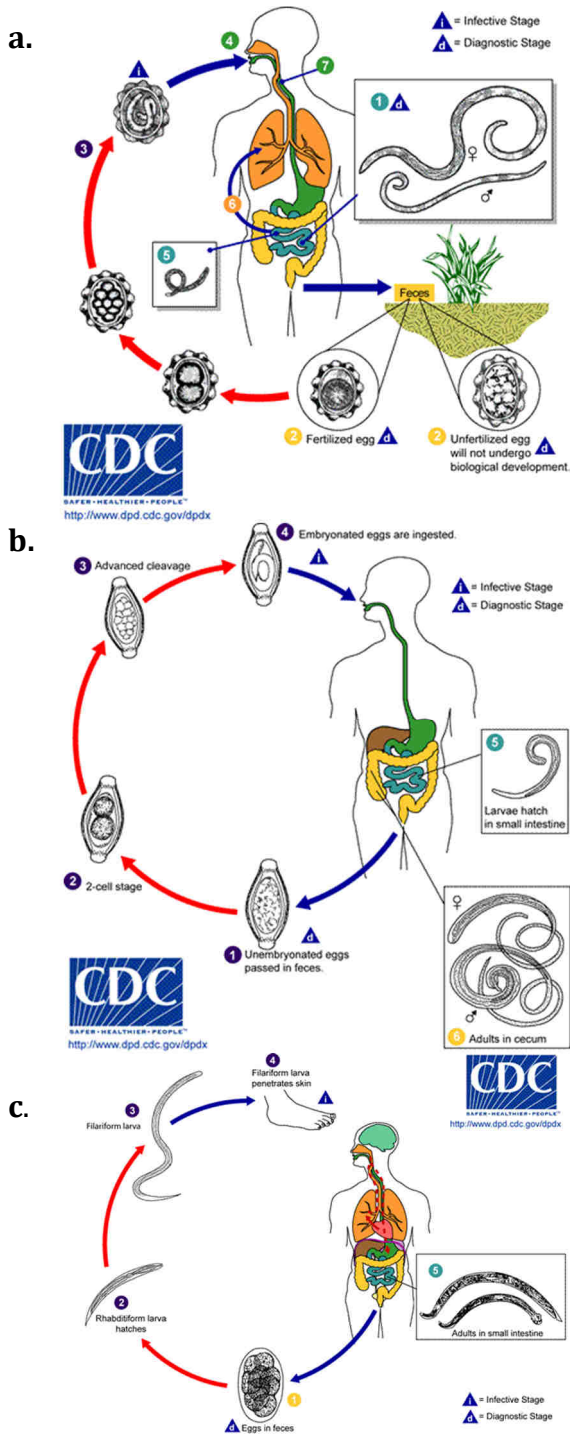


Figure 2. Life cycle of *Ascaris lumbricoides* (a), *Trichuris trichiura* (b), and hookworm (c).

The life cycles of the three STHs are shown in Figure 2. They all have a direct life cycle which means they only require one host, i.e. predominantly a human host in their case. The adult worms inhabit the intestines of the human host, where sexual reproduction takes place with the production of eggs. These eggs leave the human host in the stool and become infective as they mature in the soil for a certain period of time. However, there are some differences between the three STHs. Humans are infected by *A. lumbricoides* and *T. trichiura* by the ingestion of the eggs. Hookworm eggs first hatch in the soil and the larvae infect the human host by penetrating the skin. The larvae of *A. lumbricoides* and hookworm both have a developmental phase in the lungs of their host while the *T. trichiura* larvae only reside in the intestines.

Figure 3 shows that STHs are highly prevalent in Sub-Saharan Africa and parts of Asia. Moderate and low prevalences are found in North Africa, Latin America and other parts of Asia. STHs are non-endemic in Europe, the USA, Canada and Oceania.

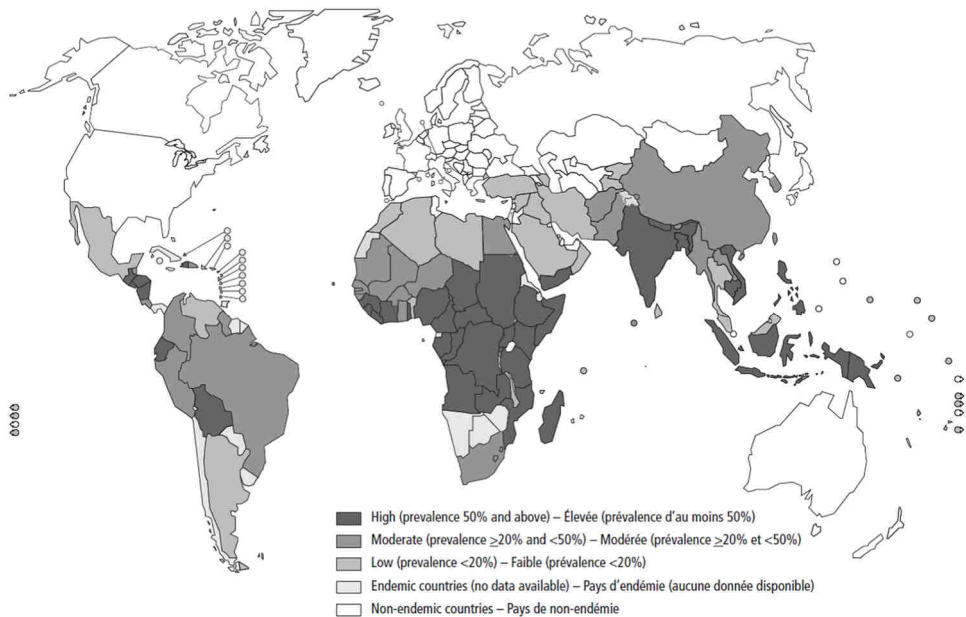


Figure 3. Global distribution of soil-transmitted helminths in 2008 (Reproduced with permission from (34)).

As shown in Figure 4a, the prevalence of STHs usually rises in childhood and becomes stable in adulthood (35). However, morbidity is related to the intensity of infection and the populations with the highest infection intensity are most vulnerable (32, 35). As shown in Figure 4b, the highest intensities are reached for *A. lumbricoides* and *T. trichiura* in children between 5 and 15 years of age while for hookworm intensity increases with age until adulthood and then reaches a plateau. Whether the drop in intensity in the first two STHs is related to less transmission, acquired immunity or both is still largely unknown (32).

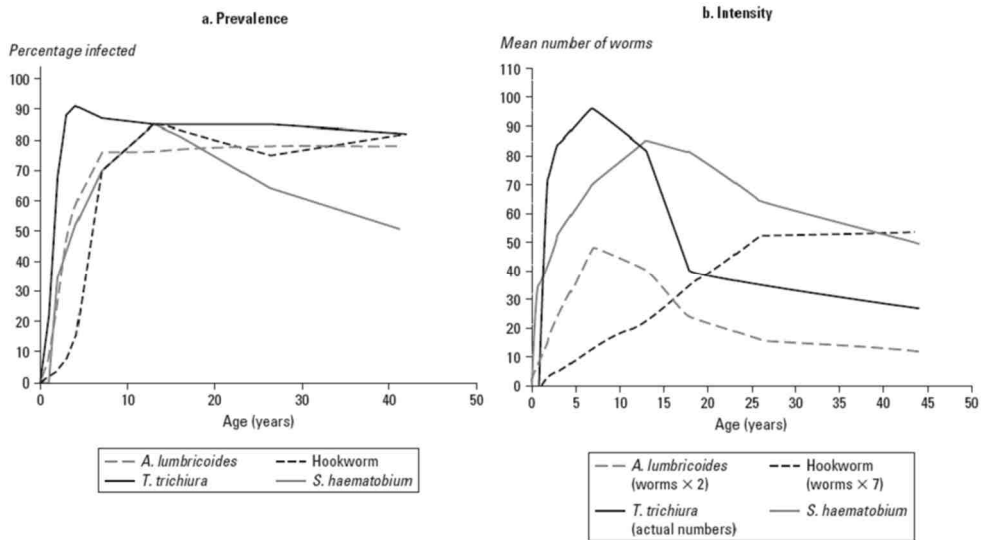


Figure 4. Age patterns in prevalence (a) and intensity (b) of STH infections (Reproduced with permission from (35)).

STHs are characterized as being highly aggregated or overdispersed in endemic populations meaning that most individuals only harbour a few worms while only a few individuals harbour many worms (32, 35). In general, 20 percent of the human population harbours approximately 80 percent of the worm population (35). Consequently, these heavily infected individuals concurrently have the highest risk on morbidity and are the major source of environmental contamination (35).

Hygiene hypothesis

In the last decades two contrary trends were observed worldwide but especially in affluent countries; while infectious disease incidence dropped, the incidence of immune disorders rose. As an example the development of infectious diseases and immune disorders in the USA is shown in Figure 5. These opposing trends are assumed to be causally associated (36). The general theory behind this association is the so-called hygiene hypothesis which postulates that childhood infections can reduce the tendency to develop allergic diseases/ immune disorders (25).

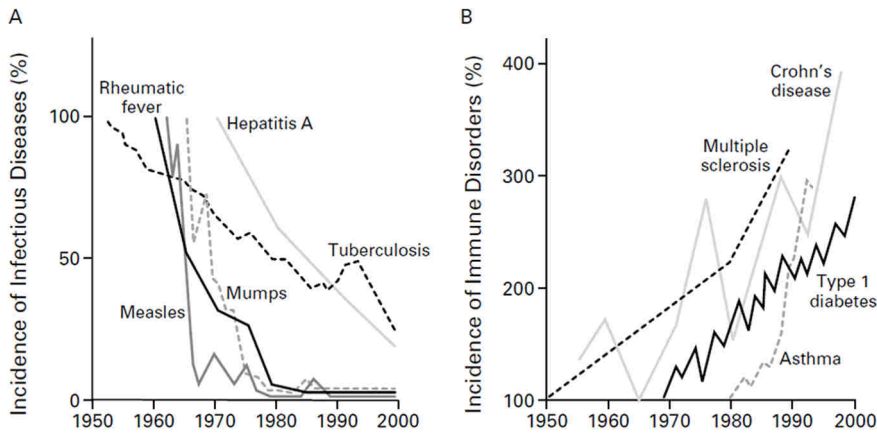
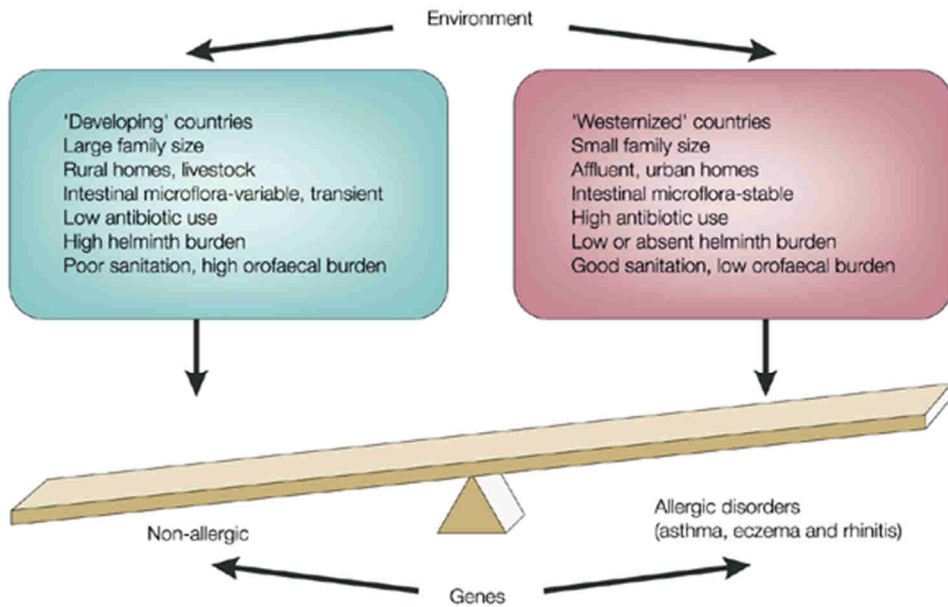


Figure 5. Trend in infectious diseases (A) and immune disorders (B) in the USA (Reproduced with permission from (36)).

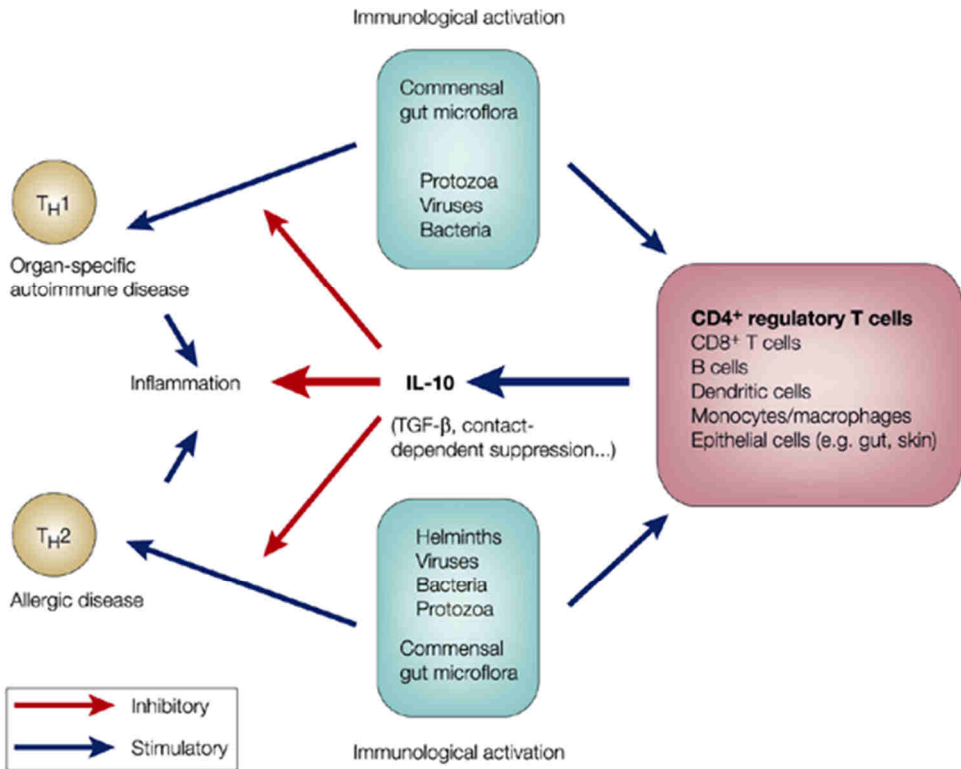
The hygiene hypothesis was first put forward in an article by David Strachan in 1989 (37), although the idea of an association between infection and immune disorders had already been discussed before (38, 39). He found that in British children a larger household, especially a higher number of older siblings, was associated with less hay fever. He linked this with early childhood infections (“... could, however, be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children.”). Thus, improvements in hygiene and societal changes in the last century like improved sanitation, reduced family size, introduction of antibiotics and vaccination, and cleaner homes have reduced the childhood exposure to infections. As a consequence the immune system does not develop properly which increases the risk of developing immune disorders like allergy (25, 26, 40). Figure 6 depicts the general idea of the hypothesis. The hypothesis has been the focus of much epidemiological and immunological research to explore the association between the environment and allergy development.



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Figure 6. General idea of the hygiene hypothesis (Reproduced with permission from (25)).

Figure 7 describes the most accepted immunological model for the hygiene hypothesis. In short, the development of immune disorders is assumed to be the result of the balance between the T helper 1 (Th1) and T helper 2 (Th2) response and the presence of regulatory T (Treg) cells. Treg cells produce IL-10, an immunosuppressive cytokine, which down-regulates Th1 and Th2 responses. Infections give a Th1 and/or Th2 response and Th1 predominance can lead to auto-immune disorders while Th2 predominance can lead to allergy. However, these infections also give rise to development of Treg cells. These cells, by producing the above mentioned IL-10, inhibit the Th1 and Th2 responses in the body and thus protect against the development of immune disorders (25, 26, 41, 42). Hence, in a hygienic environment few childhood infections occur and Treg cells hardly develop which gives rise to a Th1 or Th2 predominance, resulting in immune disorders. In an unhygienic situation many childhood infections occur and consequently many Treg cells develop which suppress the Th1 and Th2 response, and protect against the development of immune disorders (25, 26).



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Figure 7. The immunological model behind the hygiene hypothesis (Reproduced with permission from (25)).

The immunological model provides a useful framework and possible explanation for the assumed association between childhood infections and allergy/ immune disorders. It has spurred much epidemiological research into the association between the child's environment, with a particular focus on childhood infections, and the occurrence of allergy/ immune disorders. Nevertheless, although the evidence for the hypothesis is intriguing, especially on immunological grounds, the causal association has still not been unambiguously demonstrated in epidemiological research and therefore the hypothesis remains controversial (43, 44). Moreover, the hypothesis does not give an explanation for some paradoxes, e.g. that asthma is increasing in 'unhygienic' American inner cities, that infections with airborne viruses are not protective, that the inverse association between some infections and allergy are not always reproduced, and that probiotics are not effective in the prevention and therapy of allergy (45).

Next to infection also other changes occurred in the last decades, e.g. changes in diet and physical activity, which instead of or together with infections could account for the increase in allergy (46). As a consequence of the controversy, the association between childhood infection and allergy/ immune disorders is still the subject of considerable study.

Soil-transmitted helminths and allergy

Several reasons exist for the potentially important role that helminth infections and especially STH infections play in the development of allergy. Firstly, they are highly prevalent in non-affluent and rural populations while the opposite is true in affluent and urbanized populations (32, 47). Secondly, they are the most common and persistent of all childhood infections and continuously infect children living in endemic areas from soon after birth throughout childhood (31, 35). Finally, they have a strong regulatory effect on the immune system to ensure their survival within the host for several years (32, 48).

So far, research on the relationship between STHs and allergy has provided conflicting evidence, with studies showing that STH infections either promote, inhibit, or are unrelated to allergy (49-56). Some of these contradictory results could perhaps be attributed to differences in study design (e.g. adults vs. children, case-control vs. cross-sectional). However, there are also indications that the effect of STHs on allergy is influenced by several other factors, namely timing, infection intensity, and type of parasite (26, 57-60).

The timing of the STH infections is considered very important in determining the effect on allergy. This timing consists of two components, namely duration (i.e. acute vs. chronic infection) and time frame (early vs. late infection). The regulatory effect of STH infections on allergy might only be exerted in case of chronic infections, while acute infections may enhance allergy (26, 57-60). Moreover, infections early in childhood might be more beneficial for a regulatory effect than infections later in childhood. Early in childhood there might be a critical time window for the development of a non-reversible anti-allergic phenotype of the immune system. Infections experienced during that period lead to a permanent protective effect. Late infection may be protective by a reversible anti-allergic mechanism or risk enhancing by promoting Th2 predominance (58, 60). Secondly, the burden of infection is considered an important determinant of the effect of STH infections on allergy. High intensity infections have a strong regulatory effect on the immune system which suppresses allergy while low intensity infections have no regulatory or even an inflammatory effect (26, 58-60). Thus, the lowest rates

of allergy are expected to occur in school-aged children with the highest infection burdens. Moreover, these infections probably occurred early in life and remained throughout childhood (57). Finally, there are strong indications that different STHs exert a different effect on allergy (59-62). While these factors together provide a useful framework for understanding the contradictory observations in the association between STHs and allergy, they are mostly based on speculation and cross-sectional research. Longitudinal studies can provide more insight into these apparent difficult associations. However, these are still relatively scarce. Therefore, this thesis will focus on the longitudinal association between STHs and allergy.

Aim

The general aim of this thesis is to assess within epidemiological studies the longitudinal effect of STHs on allergic sensitization and atopic diseases (allergy).

Research setting and methods

The epidemiological studies described in this thesis used data obtained in a cohort of Cuban schoolchildren. Cuba provides a unique mix of characteristics from the non-affluent as well as the affluent world. Cuba is a poor country that is strained by economic hardship (e.g. US blockade). Nonetheless, the country has an excellent health service (63). Asthma levels are high in Cuba which is in contrast with the general trend in non-affluent countries (64-66). Nevertheless, like non-affluent countries, Cuba is endemic for STH infections (67-69). Taken together, this makes Cuba an interesting country to study the epidemiological association between STHs and allergy.

The study cohort consisted of school-aged children from two Cuban municipalities, namely San Juan y Martínez (SJM), in Pinar del Rio, a province in the west of Cuba, and Fomento in Sancti Spiritus, a province in the centre of Cuba (see Figure 8). The baseline cross-sectional study (P0) was performed in 2003/2004 in 1321 children aged 4-14. They were recruited from 19 randomly selected rural and urban primary schools; 5 in SJM ($N_{\text{children}}=398$) and 14 in Fomento ($N_{\text{children}} = 914$).



Figure 8. Map of Cuba with the location of the municipalities involved in the study.

Current infections with STHs in children were determined by means of stool examination which consisted of direct smear and Kato-Katz examinations. Children infected with STHs were treated with a single dose of 500 mg mebendazole. A questionnaire about parasitic infection risk factors was assessed in the child's parents or guardians. For the determination of allergy and its risk factors the ISAAC methods were followed; the child's parents or guardians were interviewed using a standard questionnaire and children were subjected to skin prick testing. Detailed information about the measurements is provided in the respective chapters.

All STH positive children in both municipalities were followed up every six months for two years (2004-2006). For ethical reasons inclusion of an untreated control group was not possible. Alternatively, in SJM at every follow-up measurement other primary schools were selected in the same way as the baseline schools. From these schools all STH positive children were selected as reference group and subjected to the same measurements as the cohort population at baseline. Assuming that each group is representative of the population of schoolchildren in SJM, the STH positives within these groups served as an approximation of a group of untreated children.

In 2007, all children of the original cohort of 2003/2004 which could be traced were measured again ($N=1136$). A flow diagram of the study is presented in Figure 9.

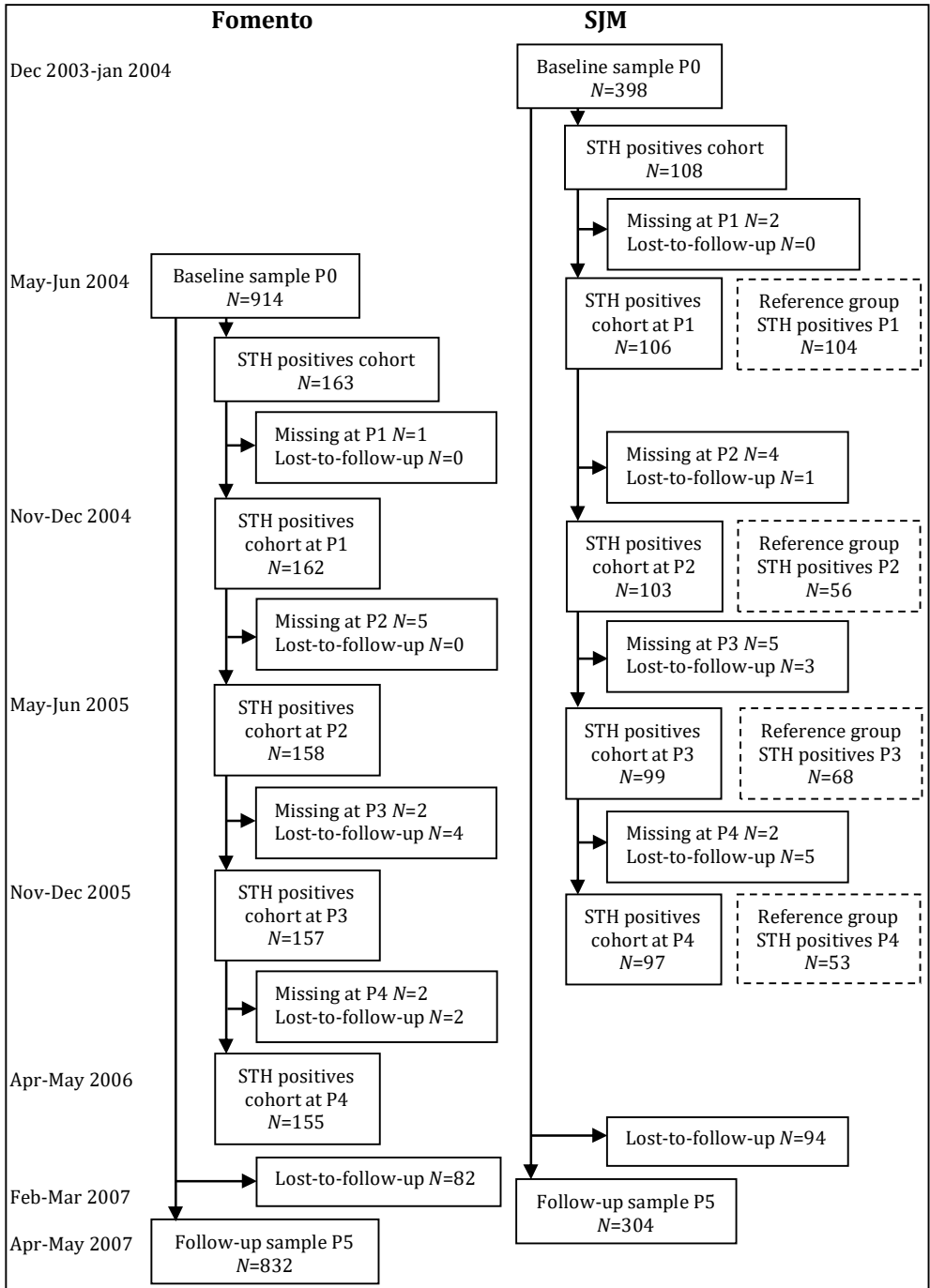


Figure 9. Flow diagram of the study population.

Outline of this thesis

To gain a better understanding of the longitudinal effect of STHs on allergy five studies were performed. These studies comprise the next five chapters.

Periodic selective treatment with a single dose of 500 mg mebendazole is one recommended treatment for the control of STHs. The effectiveness of this treatment regime in reducing STH infections is evaluated in **Chapter 2**.

In **Chapter 3**, the effect of deworming/ anthelmintic treatment on the development of allergy is assessed, using the treatment regime evaluated in Chapter 2.

The effect of STH (re)infections after deworming on allergy development is investigated in **Chapter 4**. Additionally, it is assessed if the three STH species exert a different effect on allergy development.

Next to STHs also other factors are considered important for the development of allergy. In **Chapter 5**, a prediction model is used to assess which factors of a set of common risk factors are predictors for the development of asthma.

A difficult economic period called the 'Special Period' occurred in Cuba. The effect of the economic circumstances in early childhood during this period on atopic disease occurrence, and the possible effect of factors related to this period, such as infectious diseases and undernutrition, are the focus of **Chapter 6**.

In the final chapter, **Chapter 7**, the results of the previous chapters are put into context, methodological considerations are discussed, implications are considered and future directions for research are given.

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