General discussion

As described in Chapter 1, atopic diseases are an important childhood health problem worldwide. The prevalence has increased in the past decades and atopic diseases pose a significant burden on the individual patient, family, healthcare services, and society. The observation that atopic diseases are very common and soil-transmitted helminth (STH) infections relatively uncommon in affluent and urbanized populations while the opposite is true in populations of developing countries and rural areas, has led to the hypothesis that the two phenomena may be causally associated. While this association has been studied extensively in cross-sectional research, longitudinal studies are still sparse. This thesis focusses on the longitudinal effects of STH infections on allergic sensitization and atopic diseases (i.e. allergy). To this end, we conducted a series of epidemiological studies in a cohort of Cuban schoolchildren. Firstly, we evaluated the effectiveness of periodic selective treatment with a single dose of 500 mg of the anthelminthic drug mebendazole in reducing STH infections (Chapter 2). Next, we assessed the influence of this deworming on the development of allergy over a period of two years (Chapter 3). Then, we assessed if changes in STH infection status, resulting from deworming and (re)infection, affected the risk of allergy development and if these were STH species-specific (Chapter 4). Next, we determined which risk factors, including STH infections, predicted the development of asthma (Chapter 5). Finally, we investigated the influence of early childhood exposure to the Cuban economic situation in the nineties on atopic disease occurrence later in life, and the possible effect of factors related to that period, such as infectious diseases (Chapter 6).

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In this final chapter the main findings are summarized and reflected on in a broader perspective. Also, some methodological issues are discussed. The chapter concludes with implications and future directions for research.

Main findings

We found that periodic selective deworming in Cuban schoolchildren was effective in reducing STH infections. However, periodic deworming did not lead to more atopic diseases and might even decrease asthma. Allergic sensitization on the other hand increased after deworming but this increase was only temporary. These effects appeared to be STH species-specific; (re)infection after treatment with *Ascaris lumbricoides* and *Trichuris trichiura* was associated with the development of atopic diseases while (re)infection with hookworm tended to be protective. For the development of allergic sensitization opposite effects were observed, i.e. protective for *A. lumbricoides* and *T. trichiura* and risk increasing for hookworm. Our prediction model for the development of asthma indicated no significant role for STH infections; only antibiotics use, family history of atopic diseases and allergic sensitization were

identified as predictors in our study population. Children who were exposed to poor economic circumstances during infancy and early childhood experienced less atopic diseases later in childhood than children who were not exposed. These results suggest that factors related to this period, such as increased levels of infectious diseases, may have a protective effect on atopic disease development.

Methodological considerations

All studies in this thesis were conducted in a cohort of Cuban schoolchildren. The high quality education in Cuba is compulsory and free for all children aged 6 till 15 (1). Hence, education does not depend on socioeconomic status or other factors. The Cuban schoolchildren included in our study were from randomly selected schools. There were no exclusion criteria and virtually all parents gave informed consent for their child to participate in the study. In Cuba the health system has a high standard with free of charge universal access (2-4). The socialist system in Cuba has created comparable socioeconomic circumstances for the majority of the population (5, 6). Due to the homogeneity of the population, the equitable socioeconomic conditions and the access to quality health care, related confounding factors are expected to be less influential than in most other countries. The study had a longitudinal design with a duration of three years. The response rate for all measurements was high (80-100%) and loss to follow-up low (13%). Nevertheless, there are some limitations to be considered.

For three years, all children who were STH positive at baseline were followed up every 6 months and treated if still or again positive. Ideally, a 'control cohort' with untreated STH positive schoolchildren should be followed up as well. For ethical reasons, this was not possible. However, by using the alternative of four consecutive groups of STH positive children, which are each representative of the primary school children in the municipality concerned, a longitudinal study has been approximated as much as possible. However, we cannot completely rule out other variations in STH infection, allergic sensitization, and atopic diseases between these groups than 'general time trends' only.

Due to the above mentioned random selection of schools and inclusion of nearly all children from these schools, selection bias at baseline is very unlikely. However, drop out during the follow-up period of the study can still create bias if this drop out is selective. During the entire study period in the STH positive follow-up cohort of SJM and Fomento just nine and six children, respectively, dropped out of the study. However, of the total study population at baseline (i.e. STH positives and negatives; *N*=1312) 176 children were lost-to-follow-up at the last follow-up measurement. These children differed in age, municipality, area of residence, and infection status from the children

who remained in the study, creating selective missing. This could have introduced bias in our prediction model which used this total study population data and we therefore applied multiple imputation (see Chapter 5). The prediction model with and without the imputated cases resulted in the selection of the same risk factors. Still, we cannot totally exclude bias due to loss to follow-up.

For the diagnosis of allergy standard methods of the ISAAC study were followed (7). Allergic sensitization was based on skin prick testing with seven common allergens and the ISAAC questionnaire was used for the diagnosis of atopic diseases. The skin prick test is an objective measurement and was performed by a trained nurse. Therefore, bias in the assessment of this variable is expected to be negligible. Questionnaires have inherent limitations which could have biased the diagnosis of atopic diseases. However, this bias is most likely non-differential as we do not expect it to be related with infection status. Furthermore, the ISAAC questionnaire is validated and has become the standard diagnostic method in the epidemiology of childhood atopic diseases worldwide which increases the validity and comparability of the measurements between studies/countries. Still, the non-differential bias could have led to an underestimation of the associations found.

STH infection was diagnosed by one direct smear and two 25 mg Kato-Katz examinations which were performed in one stool sample per individual. This combined with the low intensity of infection may have resulted in a reduced sensitivity of the diagnostic tests (8). However, the bias is non-differential as it is unrelated to allergy status. Therefore, the treatment effect might have been overestimated and the associations might have been underestimated. On the other hand, most other studies evaluating the efficacy or effectiveness of anthelminthic drugs are based on only one stool sample as well, thus allowing comparison between studies.

Several covariates were assessed in the study which could be potential confounders and effect modifiers. Although we adjusted for them, we cannot exclude that other unknown or unmeasured factors may have influenced the study results. Furthermore, these covariates were all questionnaire-based comprising the inherent limitations already mentioned. However, unlike atopic disease, it is likely that the bias in this case is differential, as parents or guardians of children with atopic diseases and STH infections might recall variables differently than parents or guardians of healthy children. Therefore, associations between these diseases and covariates might have been underestimated or overestimated and consequently the relationship between STH infections and atopic diseases might not have been optimally adjusted.

Research findings: reflections, interpretations and gaps

Deworming

Worldwide more than two billion people are infected with STHs, most of which are school-aged children (9-12). The impact of infection is also highest in this age group (9, 10). The current strategy to control these infections in endemic areas is periodic deworming with single dose anthelminthic drugs (9, 13). Mass treatment is recommended in moderate and high endemic areas and selective treatment in low endemic areas. Various papers have reported on the effectiveness of mass treatment, mainly in high endemic areas in Africa. However, the impact of selective treatment has so far been understudied. Nevertheless, in Latin America and Asia many areas with low endemicity for STHs, and thus target areas for selective treatment, exist. Moreover, with the ongoing mass deworming campaigns in Africa, such low endemic areas are expected to arise in that continent as well (14, 15). To anticipate an ensuing shift from mass treatment towards selective treatment strategies, more information about the effectiveness of selective anthelminthic treatment is timely and essential. In our study in Chapter 2 a biannual selective treatment regime of 500 mg mebendazole appeared effective in reducing the percentage of STH infections in a low endemic setting and equally effective as periodic mass treatment in high endemic areas (16).

Selective treatment requires the selection of STH positive individuals by individual diagnosis, which is considered more time consuming and less cost-effective, and thus a major drawback as compared to current mass treatment strategies (9). Our prediction model (Chapter 5) indicated which factors were important for persistent infection in our study population, namely male gender, sanitary disposal in latrine or open-air, and playing in the soil. More of such prediction models in other populations would be useful, as they can help identify subpopulations of children most at risk to remain infected. By targeting these specific subgroups, e.g. only children with a latrine or no sanitary facilities at home, and not all schoolchildren, selective treatment can become more time- and cost-effective and thus a more attractive alternative for mass treatment.

It is unclear which parameters are most suitable to assess the effectiveness of treatment. CR and ERR are usually assessed to determine efficacy (17). However, parameters for effectiveness are not clearly defined. Most effectiveness studies report prevalence and/or intensity or the reduction in these parameters. Some only or also report CRs and/or ERRs. In this thesis, we reported all parameters. Clearly defined parameters to assess effectiveness would benefit the performance and comparability of studies.

Although ERR is considered important for assessing drug efficacy, and could also be important for effectiveness, this parameter is rarely used. This is a serious lack as previously discussed by Keiser and Utzinger (18). In addition, no consensus exists on the population in which to calculate ERR. Sometimes it is calculated only in children who remained positive after treatment and other times in all children who received treatment, i.e. positive and negative children after treatment. Furthermore, ERRs can be based on arithmetic or geometric means or medians. These differences make comparison between studies and subsequent interpretation of ERRs difficult. Consensus about the method for calculating ERRs would be very valuable.

Only for ERR thresholds are defined, not for CRs or PRRs. For drug efficacy an ERR threshold of 70% for *A. lumbricoides* and of 50% for *T. trichiura* are accepted (17). No threshold is defined for ERR in hookworm. However, with clear thresholds for all relevant parameters and all STHs the efficacy and effectiveness of treatment programs can be better assessed.

Vercruysse *et al.* (19) suggested that for albendazole the thresholds should be increased to an ERR of 95% for *A. lumbricoides* and of 90% for hookworm. A standard threshold for *T. trichiura* was not defined as a single-dose of albendazole is unlikely to be satisfactory against infections with this helminth. Based on the results of the present study, the thresholds for mebendazole against *A. lumbricoides* and *T. trichiura* may have to be updated as well and, at least for efficacy studies, be set at 95% and 90%, respectively. Mebendazole is less efficacious against hookworm and therefore the situation that no threshold is defined could be continued. However, as mentioned above, thresholds for all STHs would be useful and the ERR threshold for hookworm could at minimum be set at 60%. For effectiveness studies slightly lower thresholds should be considered for all STHs as real life circumstances might reduce the treatment effect.

Influence of deworming on allergy

STH have been linked with allergy through the hygiene hypothesis. This hypothesis assumes that experiencing childhood infections is important for the development of a balanced immune system which protects against the development of allergy. If this hygiene hypothesis is true then deworming might lead to the development of or an increase in allergy.

In murine models evidence exists of a protective effect of helminth infections on allergy (20-23). However, despite a body of suggestive observations, an inverse association between helminth infections and allergy in humans has so far not been conclusively

established (24-26). Previous cross-sectional studies have provided conflicting evidence showing that helminth infections either cause (27-29), inhibit (30, 31), or are unrelated to allergy (32, 33), or that allergy inhibits helminth infections (34). Similarly, our longitudinal studies do not provide strong evidence in favour of the hypothesis that STH infections protect against the development of allergy. We observed no negative effects of deworming on atopic diseases and possibly even a moderate beneficial effect on asthma. Among the few longitudinal intervention studies performed by others, the majority showed no detrimental effects of deworming on atopic diseases either (35-37); one showed a beneficial effect on asthma (38). Only one study showed an increase in atopic dermatitis (37). Our deworming study did show a –temporary- increase in allergic sensitization (39), in line with other longitudinal intervention studies (36, 37, 40, 41). One study did not find an effect of deworming (35) and another observed a decrease in allergic sensitization (38). In other words, the hypothesis that helminths have a protective effect on allergy could not be corroborated by longitudinal studies either. Apparently, the relationship between helminth infections and allergy is much more complex than assumed. The contradictory results have been attributed to several factors, such as timing, infection intensity, and type of parasite (see also Introduction) (42-46). Below, we will discuss these in more detail and in relation to the studies in this thesis.

Timing of infection

Timing of infection concerns the time of first infection and the duration of infection (43). Infections in the first years of life may have a different effect than infections later in childhood or adulthood. Likewise, acute infections -primary infections and repeated or intermittent infections- may exert a different effect than chronic infections -continuous/long-lasting infections. The hypothesis is that early and/or chronic infections down-regulate allergic responses while late and/or acute infections may increase allergic responses (43-45). Our study (39) as well as other longitudinal intervention studies (35-38) were performed in schoolchildren and therefore give us only an indication of the effect of late childhood STH infections on atopic diseases prevalence. Most of these studies showed that clearance of STH infections by deworming did not increase atopic disease prevalence, suggesting that there is no major effect of late childhood infections. However, our and one other study showed that deworming of schoolchildren might decrease asthma (38, 39) which suggests that if late infections do have an effect that they might indeed increase atopic diseases. The results of Chapter 4 suggested that (re)infections after anthelminthic treatment, which can be considered ‘acute’ infections, increase the occurrence of atopic diseases. Longitudinal

intervention studies in preschool children are still lacking, and therefore the effect of early infections remains undetermined. However, our study on the impact of the Cuban 'Special Period' showed that poor economic circumstances during infancy and early childhood had an attenuating effect on atopic disease occurrence later in childhood (47). Increased infection levels have been related to this period, suggesting that early infections may be involved in atopic disease development.

The picture for allergic sensitization looks different from the one for atopic diseases. Our study together with other longitudinal intervention studies indicates that deworming might, albeit temporarily, increase allergic sensitization (35-41). This suggests that late childhood infections have a beneficial effect on allergic sensitization. This was also found by Rodrigues *et al.* (48). However, their main finding was that especially early infections decrease allergic sensitization.

Infection intensity

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 (42-44, 46). As our study population had predominantly low intensity infections, we could not explore this supposed effect of infection intensity. To our knowledge no other studies apart from Rodrigues *et al.* (48) have studied the effect of STH infection intensity in humans, and their results suggest that especially high intensity infections decrease allergic sensitization.

Species-specificity

The results of Chapter 4 that acute infections might increase atopic diseases mainly concerned *A. lumbricoides* and *T. trichiura*. Acute hookworm infections on the other hand appeared to decrease atopic diseases. A meta-analysis of cross-sectional studies also indicated that especially hookworm might have a protective effect on atopic disease development while *A. lumbricoides* and *T. trichiura* might not have this protective effect (49). This suggests that the species of STH could be more important than the timing of infection. It is not clear why hookworm might be different from *A. lumbricoides* and *T. trichiura* in their effect on atopic diseases. However, if STH species indeed have different effects, hookworm affects the immune system in another way than the other two STHs. In Chapter 1 the immunological background for the hygiene hypothesis is explained. This model, where the balance between the T helper 1 (Th1) and T helper 2 (Th2) response and the presence of regulatory T (Treg) cells

determine the development of immune disorders, is still the dominant model for the mechanism behind the hygiene hypothesis. However, with the discovery of T helper 17 (Th17) cells, which are involved in the inflammation process, another model has been developed (see Figure 1), but this is not yet widely applied. In this model the balance between Th1/Th2 and between Treg/Th17 determines if and which type of immune disorders can develop or not, and the development of Treg or Th17 cells depends on how well a helminth is adapted to its host, i.e. promoting its own survival in the host without impairing the hosts' survival (50). During infection with a well-adapted helminth, Treg cells are developed and probably no immune disorders occur while with a poor-adapted helminth, Th17 cells are developed and immune disorders could occur. When combining this new model with our study results on atopic diseases, this could mean that hookworm is better adapted to the human host than *A. lumbricoides* and *T. trichiura*. Hookworm has a longer life span in the human host (5-7 years) than *A. lumbricoides* and *T. trichiura* (1-2 years) (10), which might imply that hookworm has (developed) a better immune suppressive response to survive in their host. Hookworm infects the host via larvae instead of via eggs in the case of *A. lumbricoides* and *T. trichiura* (10). Also, children generally acquire a hookworm infection at an older age than an *A. lumbricoides* or *T. trichiura* infection (9, 10). These aspects could lead to another immune response of the host to hookworm than to *A. lumbricoides* or *T. trichiura*. However, other mechanisms may play a role as well, and this apparent species-specific effect warrants more exploration.

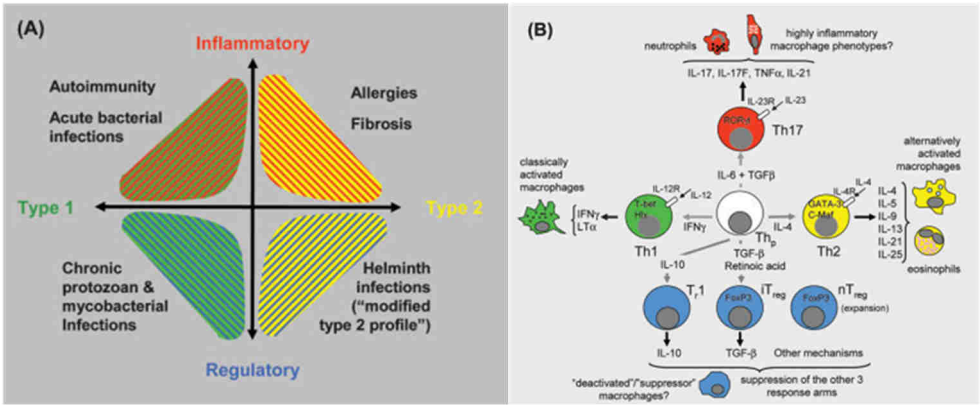

Figure 1. Map of the two axes involved in a recent model for the mechanisms behind the hygiene hypothesis (A) and the cells involved (B) (Reproduced with permission from (50)).

Atopic diseases vs. allergic sensitization

In our studies, we found that the effects of STH infections on atopic diseases were opposite to those on allergic sensitization, although the latter may only be transient. Also other longitudinal intervention studies indicate that the effect of STHs might not be the same for allergic sensitization and atopic diseases (35-41). This was also observed in two meta-analyses of cross-sectional studies on intestinal parasite infections in relation to asthma and allergic sensitization, respectively (49, 51). The first meta-analysis found that the risk of asthma was increased by STH infections, except for hookworm (49). In the second meta-analysis STH infections reduced the risk of allergic sensitization (51). At present there is no explanation for this difference in effect. However, the general assumption that STH infections exert the same effect on allergic sensitization and atopic diseases does not seem to hold. This would imply that results from studies on allergic sensitization cannot simply be extrapolated to atopic diseases and vice versa.

In the figure below an effort is made to summarize the findings and reflections regarding the relationship between STH infections and allergy as described above.

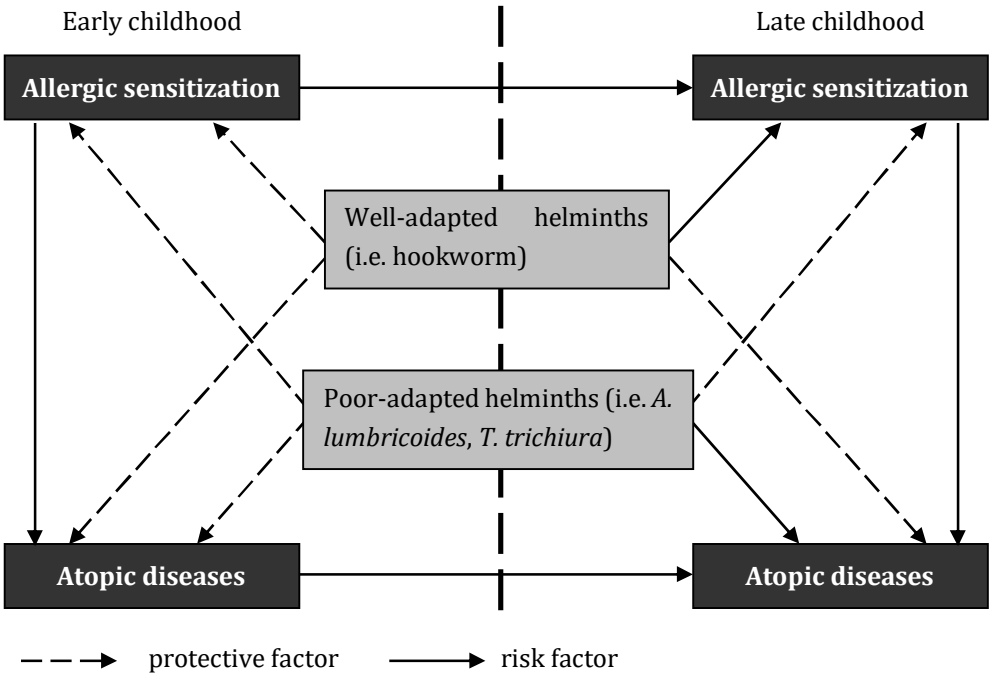

Figure 2. Effect of STHs on allergy, based on findings in this thesis.

Other factors than STH infection

In this thesis, we studied the effects of STH infections on allergy. It should be noted however that the effect of STHs alone may be marginal. Our prediction model in Chapter 5 did not show an important role for STH infections in the development of atopic diseases (52). Also, the current worldwide trends point towards a limited role of (STH) infection on allergy. While infectious disease prevalences are still decreasing, there are indications that atopic disease prevalences after decades of increase have reached a plateau and are even decreasing in some, especially affluent, countries (53-59). Preliminary results from Cuba show a decreasing trend in allergy as well as STH infections (see Table 1). These results might indicate that STH infections [1] do not have an effect, [2] only exert an effect above a certain prevalence, [3] have a limited effect, or [4] only exert an effect in combination with other factors on the development of allergy.

Table 1. Population characteristics and prevalence of allergy and STH infection in two cohorts of Cuban schoolchildren.

	Cohort 2003/2004 SJM & Fomento	**Cohort 2003/2004 SJM only**	**Cohort 2009 SJM**
N	1321	398	1389
Age (mean (range))	8 (4-14)	8 (5-13)	8 (4-13)
Sex (boys)	51.3 (678)	48.0 (191)	53.4 (742)
Living area (urban)	52.2 (690)	52.0 (207)	46.1 (641)
Allergic sensitization	20.6 (272)	11.3 (45)	11.6 (161)
Asthma	21.1 (279)	29.9 (119)	14.5 (197)
Allergic rhinoconjunctivitis	13.6 (180)	15.6 (62)	6.6 (90)
Atopic dermatitis	8.3 (110)	9.1 (36)	4.7 (64)
Any atopic disease	32.2 (426)	40.7 (162)	20.6 (280)
***A. lumbricoides* infection**	6.3 (83)	14.8 (58)	5.2 (72)
***T. trichiura* infection**	10.6 (139)	13.0 (51)	3.2 (44)
Hookworm infection	9.2 (121)	7.1 (28)	1.2 (17)
Any STH infection	20.9 (274)	27.8 (109)	8.6 (119)

Selection procedures and assessments of infection, atopic disease and allergic sensitization status were similar for both cohorts (2003/2004 and 2009).

One factor that could have an effect on allergy development next to, or in combination with STH infections is nutrition. This was suggested in our ecological study (Chapter 6) on the influence of the economic crisis in Cuba in the nineties (47). Poor nutritional status and helminth infections have a similar geographical distribution with the same individuals often experiencing both conditions concurrently (60). A vicious cycle between nutrition and infectious disease has been proposed: infection deteriorates nutritional status while a poor nutritional status increases susceptibility to infection (60-63). In this cycle there is a strong involvement of the immune system (60, 63), which in turn is related to allergy (64, 65). This triangular relationship is visualized in Figure 3. There is evidence that specific micronutrients influence parasite immunity, particularly zinc, iron, and vitamin A (66). Thus, the observed increase of allergy in affluent countries may not only arise from reduced exposure to childhood infections, but also from changed/improved availability of nutrients. Moreover, it has been postulated that diet changes, especially reduced antioxidant intake, account for the increase in prevalences of asthma and other atopic diseases in affluent countries (67-70). In addition, since prevalences of both asthma and obesity have concurrently increased in the last decades, a causal relationship has been suggested between these two phenomena, although the underlying mechanisms are still unresolved (71-76). To our knowledge no studies have investigated the concurrent effect of nutritional status and infection on allergy development.

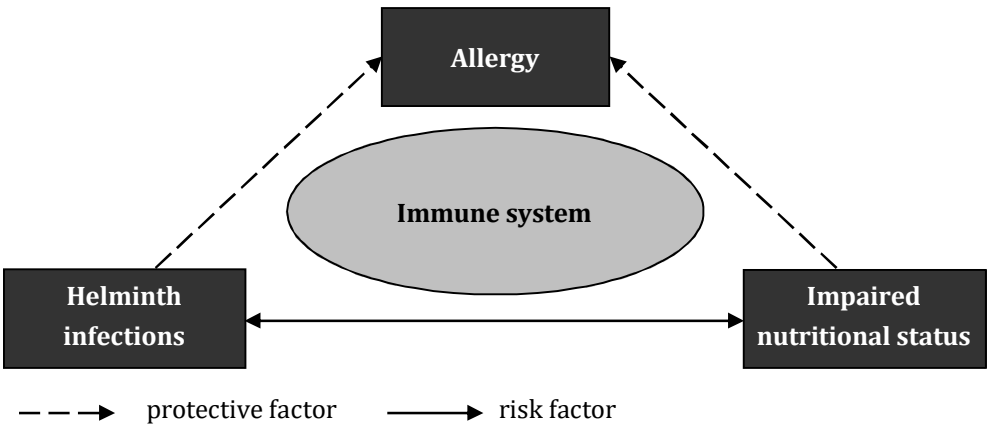

Figure 3. The triangular relationship between allergy, helminth infections, and nutritional status.

What's next

The results of the deworming study show that selective treatment is as effective as mass treatment, and that two rounds of treatment appeared sufficient to obtain substantial reductions in STH infection. Male gender and the habit of playing in the soil were risk factors for 'persistent' infection. Such information can be helpful to design selective treatment programmes in low endemic settings. Currently, well-designed studies evaluating the effectiveness of selective treatment programmes are lacking. However, more this research is needed for sound policy decisions on the most effective selective treatment regime to reduce STH infections in low endemic areas, or to further reduce STH prevalence in areas that have been undergoing mass treatment.

Based on the results of this thesis and previous research by others, deworming of schoolchildren is unlikely to promote allergy. Nevertheless, the relationship between STH infections and allergy remains inconclusive. To shed more light on this relationship and the underlying mechanisms, further research is still needed.

As described in this thesis, the effect of STHs on allergy appears to be species-specific and opposite for allergic sensitization compared to atopic diseases. Research that further clarifies the mechanisms behind these two effects would be useful.

Research on the hygiene hypothesis has focussed on individual associations of certain risk factors –including STHs– with allergy. In this way the importance of each factor relatively to other factors cannot be determined. However, allergy is multifactorial in origin, implying that prevention or control should be multifactorial as well. Hence, more prediction models should be developed to determine which (combination of) factors predict allergy development. Apart from infection, amongst others, nutritional factors, pollution, breastfeeding, smoking exposure, pet exposure, sanitation, and vaccination should be considered.

As described in this thesis nutritional status and infections share an interesting link with each other and with allergy. Hence, research on the combined effect of nutritional status and STH infection on allergy could be another interesting research topic.

While most research, including the research in this thesis, has been performed in schoolchildren, it has become clear that the strongest effect of (STH) infections and other factors is expected in early childhood. Therefore, research should focus on pre-school children. If possible, birth cohorts are even a better option. Research in these populations is often more difficult due to ethical and practical considerations, but are needed to elucidate if and to what extent (STH) infection affects allergy development.

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