Helminth infections, socio-economic status and allergies in Indonesia

Firdaus Hamid

ISBN: 978-94-6182-598-8

The research presented in this thesis was performed at the Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; the Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia & the Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

The studies described in this thesis were financially supported by the Royal Netherlands Academy of Arts and Sciences (KNAW), the European Commission (Glofal, FP6-2003-FOOD-2-B) & the European Academy of Allergy and Clinical Immunology (EAACI)

The layout and printing of this thesis were financially supported by HAL Allergy Benelux B.V., Leiden University & Thermo Fisher

Cover design: Sri Maharani Budi

The layout and printing: Off Page, Amsterdam

Helminth infections, socio-economic status and allergies in Indonesia

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van de Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op woensdag 23 September 2015
klokke 10.00 uur

door

Firdaus Hamid
Geboren te Ujung Pandang (Indonesië)
in 1977

PROMOTIECOMMISSIE:

Promotores:
Prof. Dr. M. Yazdanbakhsh
Prof. Dr. R. van Ree (Academisch Medisch Centrum, Universiteit van Amsterdam)

Copromotor:
Dr. E. Sartono

Overige leden:
Prof. dr. R. Gerth van Wijk (Erasmus Medisch Centrum, Erasmus Universiteit Rotterdam)
Dr. J. Lidholm (Immunodiagnostics Unit, Thermo Fisher Scientific, Uppsala, Sweden)
Prof. dr. L.G. Visser
Dr. E.A. van Lieshout

1 GENERAL INTRODUCTION

Partly based on:
Helminth-induced IgE and protection against allergic disorders

Firdaus Hamid[1,2], Abena S Amoah[2,3], Ronald van Ree[4], Maria Yazdanbakhsh[2]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana
4. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Current Topics in Microbiology and Immunology 2015; 388: 91-108

INTRODUCTION

Over the past few decades, the prevalence of allergic disease has been on the rise in both developed and developing countries[1]. There is strong evidence for differences in the prevalence of atopic disorders between urban and rural areas in many parts of the world, with higher prevalence of allergic diseases reported in urban areas[2-7].

The observed urban-rural differences may be influenced by socio-economic status (SES)[4]. The measurement of a child's SES can be approximated by consideration of household income, parental occupation and education level, living conditions, or habitation in geographic locations with a predominantly poor population[8,9]. These variables have each been linked with childhood asthma[9,10]. However, some studies of SES and asthma have reported contradictory results. Some have reported that children in families with low SES have an increased asthma risk[11-14], while others have found an inverse relation between low household income and asthma[15] or no relationship between asthma and parental SES levels[16-18]. These conflicting results may stem from the complexity of the meaning of SES, which can vary between studies, locations and culture[10].

Apart from exposure to allergens, altogether environmental factors that may encompass or be related to SES, along with genetics, play a key role in the development of allergic diseases. Environmental influences can include exposures to pollution, microbes, parasites and lifestyle[19,20]. Of particular interest have been infections with parasitic helminths that are highly prevalent in tropical regions of the developing world. It is estimated that a quarter of the world's population is chronically infected with helminths such as *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* or *Ancylostoma duodenale* (hookworms), schistosomes and filarial worms[21].

Despite the close parallels between immune responses that characterize helminth infections and allergic diseases, namely increased levels of immunoglobulin (Ig)-E and eosinophils along with T cells that preferentially secrete T helper type 2 (Th2) cytokines, the clinical outcome with respect to immediate hypersensitivity and inflammation is not the same[22]. Moreover, there is little geographical overlap worldwide between helminth infections and allergies. In fact, several studies have reported a negative association between the presence of helminth infections and atopic disorders[23]. However, the relationship between atopic disorders and helminth infections is not always consistent since there are also studies that show that helminth infections have no effect or are associated with increased atopic disorders[24,25].

HELMINTH INFECTIONS AND ATOPIC DISORDERS

The prevalence of allergic diseases has been increasing mainly in the developed world where changes in lifestyle and environment have taken place, characterized by increasing sanitation, hygienic measures and urbanization including pollution. In less developed countries, the prevalence of atopic disorders is relatively low but the so-called "allergic march" is starting in these geographical areas due to ongoing dramatic changes in lifestyle

and the environment. Although not as pronounced as in western countries, atopic disorders are prevalent in developing countries with the tendency for prevalences in urban centers to approach those seen in affluent countries.

In developed countries, the presence of IgE antibodies to allergens increases the risk of atopic disorders. These antibodies which bind to high affinity IgE receptors present on effector cells of the allergic response, i.e. to mast cells and basophils. These IgE antibodies can be cross linked by allergens, an event that leads to effector cell degranulation including histamine release. The mast cell degranulation leads to inflammation and if the target organ is the airways it can result in symptoms which can be recognized as an asthmatic attack. One of the diagnostic methods for allergy is to perform a skin prick test[26]. This is based on applying allergens to the skin, pricking the skin with a lancet and assessing whether a reaction develops to the allergen within 15 minutes. A wheal swelling and flare (redness) reaction induced by degranulation of skin-resident mast cells is considered as proof of sensitization to the allergen extract applied. In developed countries, the skin prick test (SPT) is often interchangeable with the measurement of IgE antibodies to allergens by *in vitro* tests[27], i.e. both tests usually show quite close correlation, with a positive test frequently (but not always!) being associated with clinical allergy. However, the relationship between IgE, SPT and symptoms of allergy can be different in different geographical areas. For example, the proportion of SPT reactivity in allergic asthma appears to be much smaller in some rural areas compared to urban centers in Europe[28] or in many developing nations compared to developed ones[29,30]. Helminth infections have some interesting effects on the relationship between IgE, SPT and clinical symptoms.

Several epidemiological studies have shown that helminth infections can be negatively associated with allergic outcomes[23]. Some studies have shown that having schistosome or filarial infections decreases the risk of SPT positivity[31,32]. Similarly, studies in Ecuadorian and Vietnamese children have demonstrated that having soil transmitted helminth infections decreases the risk of SPT positivity[33,34]. In addition, Cooper *et al.* found that the presence of serological markers of chronic helminth infections (elevated levels of total serum IgE or anti-*A. lumbricoides* IgG4) were independently negatively associated with allergen SPT reactivity[35]. Araujo *et al.* reported a strong and inverse association between skin responses to allergens and infection *with Schistosoma mansoni*, among persons living in an area endemic for this helminth[36]. Similarly, a study in Gabonese children observed that the risk of a positive SPT was reduced by 72% if a child was infected with *S. haematobium*[32]. Moreover, an investigation conducted among 1385 urban and rural Ghanaian children aged 5-16 showed a strong negative association between schistosome infection and SPT reactivity to mite but not with reported wheeze or asthma[37]. However, in regard to clinical symptoms of asthma, a case-control study in urban and rural Ethiopians aged 16 to 60 years, indicated that active hookworm infection reduced the risk of reported wheeze[38].

Although the majority of studies have shown a negative association between helminth infection and SPT, there are studies that show that helminths may increase the risk of asthma and other atopic disorders. Obihara *et al.* found *Ascaris*-specific IgE may be a risk

factor of atopic disease in populations exposed to mild *A. lumbricoides* infection[24]. In line with this, a study in China, in an area with a low burden of *A. lumbricoides*, demonstrated a positive association between helminth and allergen skin test reactivity as well as asthma risk[25]. Finally, there are also studies showing no significant association. A study in Brazil among patients aged 12 to 30 years with asthma or rhinitis living in an urban area endemic for geohelminths showed that individuals infected with a low intensity of *A. lumbricoides* did not differ in the frequency of positive SPT to dust mites from those negative for *A. lumbricoides* living in the same area[39]. A birth cohort study of children from Ethiopia, which investigated the effect of geohelminths on allergic symptoms, at 3 years of age, found that there was no association between helminth infections and wheeze nor with eczema[40].

Most studies on helminth infections and allergy mentioned above had a cross sectional design. Intervention studies using anthelmintic treatment are an important to investigate whether helminth infections play a role in atopic disorders. So far, excluding the study in this thesis, two randomised placebo-controlled trials with albendazole have been conducted but they showed conflicting results. One study conducted in Ecuador, comprising a school-cluster randomised trial of one year albendazole treatment, reported no change in either sensitization to allergens or allergic symptoms[41], while another trial in Vietnam showed that the prevalence of skin reactivity increased after one year of albendazole treatment but, consistent with the Ecuadorean study, clinical allergy did not change significantly[42]. It is difficult to explain these conflicting observations, which may relate to differences in treatment regimens (incomplete and/or short-lived helminth eradication), intensity of infection, co-infections, reinfections or acute vs chronic infections[43,44].

MECHANISMS BEHIND THE ASSOCIATION BETWEEN HELMINTHS AND ALLERGIES

Given that most studies seem to show a negative association between chronic helminth infections and SPT reactivity, researchers have been looking for the mechanisms that could explain this. Although the mechanisms associated with this inverse relationship are not fully understood, it has been suggested that strong immune regulatory networks might be involved. This means that high levels of suppressory cytokines such as interleukin (IL)-10 and transforming growth factor-beta (TGF-β) as well as regulatory T and B cells[45], which seem to expand during chronic infections with helminth parasites, might down regulate allergic responses. The question as to where in the allergy cascade they exert their down regulatory activity is still unanswered. It is possible that regulatory responses affect Th2 and thereby IgE. However, immune regulation could also down-regulate the effector phase of an allergic response which involves inflammation induced by mast cell degranulation[46]. This notion is supported by reports showing that IL-10 could inhibit basophil degranulation[47] and by the negative association between IL-10 and SPT[48,49]. Regarding antibodies, in the 1970s the idea that helminths might induce polyclonal stimulation of IgE-producing plasma cells with many different specificities that would compete with allergen specific IgE for binding

to high affinity IgE receptors on mast cells was first proposed[50,51]. This competition would reduce the chance of an allergen dependent mast cell degranulation and therefore explain the absence of strong allergic responses in helminth infected subjects[52]. However, Mitre *et al.* have shown that the high ratio of polyclonal IgE to allergen specific IgE did not inhibit basophil degranulation[53]. Moreover, it has been shown that increases in IgE result in the up-regulation of IgE receptors and that anti-IgE treatment is often accompanied by down-regulation of IgE receptors which argues against the ability of high total IgE to compete out specific IgE[54]. An alternative hypothesis that helminth infections may be associated with increased levels of allergen-specific IgE that are functionally poor and therefore cannot lead to basophil or mast cell degranulation[55] is gaining support from several recent studies that indicate cross-reactive IgE might be associated with poor biological activity[56,57].

CROSS-REACTIVITY BETWEEN ALLERGEN AND HELMINTH

In general, cross-reactivity reflects the phylogenetic relationship between organisms that results in a high degree of homology in the primary structure of proteins[58]. Cross-reactivity occurs when antibodies elicited to one epitope also recognize similar epitopes in other homologous molecules[59]. In allergy, the allergen that is supposed to induce the original allergic responses is named the primary sensitizer, and the others are considered cross-reactive allergens[59]. Two types of IgE cross-reactivities have been described, one due to homologous proteins, and the other due to sugar moieties (complex N-glycans on plant and invertebrate glycoproteins) known as cross-reactive carbohydrate determinants (CCDs)[58].

Cross-reactive carbohydrate determinants (CCDs) and helminths

The asparagine-linked carbohydrate components of plant and insect glyco-proteins are highly cross-reactive and are known as cross-reactive carbohydrate determinants (CCDs)[60]. Two typical non-mammalian substitutions to N-glycans of plant glycoproteins are an α(1,3)-linked fucose on the proximal N-acetyl glucosamine and a β(1,2)-linked xylose on the core mannose[61]. Bromelain is a protein from pineapple stem which carries N-glycan structure that is often used as a marker for CCDs reactivity.

These CCD epitopes are also found in helminth parasites. The existence of IgE antibodies directed to CCDs was first reported by Aalberse *et al.* in the early 1980s[62]. The study demonstrated that serum IgE from European pollen or venom allergic patients cross-reacted with extracts from various allergenic foods. However, treating the extracts with periodate, which destroys the carbohydrate structures abolished IgE binding, indicating the involvement of carbohydrates in this cross-reactivity[62]. Another investigation by the same group observed elevated levels of IgE against peanut extract among grass pollen-sensitized European patients without peanut SPT reactivity or clinical symptoms of peanut allergy[63]. Furthermore, among 91% of those with a discrepancy between specific IgE to peanut and

SPT, IgE against CCDs could be detected[63]. In some of these patients, almost complete inhibition of IgE to peanut (as measured by competitive radioallergosorbent test) was possible with CCD. In addition, cross-reactive IgE directed against CCDs in this study was demonstrated to have poor biologic activity in terms of inducing basophil degranulation[63].

The most convincing proof of the complete lack of clinical relevance of CCD-specific IgE was reported by Mari *et al*. in a study where grass pollen allergic patients with high titers of CCD-specific IgE were skin tested and subjected to an oral challenge with human lactoferrin expressed in rice kernels. This transgenic molecule was substituted with multiple IgE-binding glycans, but both SPT and oral challenge were completely negative[64]. Therefore, this study shows that CCDs, although having (poor) biological activity (histamine release from basophils) this does not translate into clinical allergy. It has not yet been elucidated what the explanation of this lack of clinical relevance is, but the polyvalent conjugation of lactoferrin with CCDs demonstrates that it cannot simply be explained by monovalency.

With regard to helminth-induced IgE cross-reactivity, a recent study among schoolchildren (aged 5-16 years) in Ghana, West Africa demonstrated how cross-reactivity between helminth antigens and allergens can affect IgE sensitization patterns and clinical expression of allergy[65]. In this study the overall prevalence of peanut-IgE sensitization was 17.5% (233 out of 1328). However, none of the peanut-IgE sensitized children had either SPT reactivity to peanut or reported adverse reactions to peanut. In this study, the presence of *S. haematobium* infection was positively associated with an increased risk of having peanut specific IgE. In a subset of this study population, both the CCD marker bromelain and *S. haematobium* soluble egg antigen (SEA) inhibited IgE binding to peanut extract. This study also showed that peanut-specific IgE was strongly correlated with CCD-specific IgE. These results indicate that much of IgE to peanut in Ghanaian children could be directed against CCD which is also present in the schistosome SEA. The basophil histamine release assays demonstrated that the IgE directed against peanut in this population had low biologic activity[65].

This study provides a model which proposes that parasite-induced IgE against CCDs that are carried by parasites, might account for high IgE levels to food allergens and the finding that this IgE does not lead to reactivity to allergenic extracts either *in vitro* (in basophil release assay) or *in vivo* (in skin prick testing) further confirms that these IgEs to CCDs are clinically irrelevant[66].

Peptide cross-reactivity and helminth

Cross reactions between allergens from invertebrates such as for example mite and snail; cockroach and *Ascaris*; mite, shrimp, and cockroach; and mites and schistosomes[58] have been reported and involve protein cross reactivity. Three of the proteins that are involved in these examples are tropomyosin, glutathione S-transferase (GST) and paramyosin[58].

Tropomyosins are proteins involved in the contraction of muscle cells along with actin and myosin[67]. Not only are tropomyosins major allergens of seafood, mite and cockroach but are also highly immunogenic helminth proteins[68]. Tropomyosins from invertebrates

are strong inducers of IgE antibody responses in humans[69]. Santiago *et al*. demonstrated that there is 72% identity at the amino acid level between the tropomyosin from the filarial parasite *Onchocerca volvulus* (OvTrop) and the house dust mite tropomyosin Der p 10[70]. They showed a strong correlation between specific IgE to Der p 10 and IgE to OvTrop. In addition, histamine release from basophils sensitized with the sera of individuals IgE-positive to Der p 10 could be triggered by either the OvTrop or Der p 10. It is however important to realize that such biological activity is not a proof of clinical allergy. In the study by Mari *et al*. with transgenic lactoferrin, histamine release was reported at relatively high protein concentrations, but SPT and oral challenge with the same molecule were negative[64]. The study by Santiago *et al*. does however confirm that the anti-tropomyosin antibodies induced in filarial infection are cross-reactive with those allergenic tropomyosins of invertebrates (mite) that may affect sensitization[70]. As expected, no clinical mite allergy was reported for the subjects studied, indicating that these cross-reactive responses are of no clinical relevance.

In another investigation, Santos *et al*. showed that the predicted structure of *A. lumbricoides* tropomyosin was similar to that of *Periplaneta americana* tropomyosin[71]. The same study compared IgE responses to these proteins in Brazilian children aged 3-6 years living in an area endemic for helminths to responses in cockroach allergic patients aged 2 to 52 years also from Brazil[71]. A strong correlation was also found for IgE antibodies to tropomyosin from *A. lumbricoides* and from *P. americana* in sera from both populations. Seventy-six percent (90 out of 119) of subjects from the parasite endemic area had positive IgE antibodies against cockroach tropomyosin without allergy to cockroach[71]. A study conducted by Acevedo *et al.* has also demonstrated high allergenic cross-reactivity between *Blomia tropicalis* tropomyosin (Blo t 10) and *Ascaris* tropomyosin in Colombian asthmatic patients[72].

The glutathione S-transferases (GSTs) are detoxification enzymes found in most living organisms[73]. The important known sources of GSTs are cockroaches, house dust mites and molds, however, those GSTs from invertebrates including helminths are known to be strong inducers of IgE[74]. Moreover, *Blattella germanica* GST caused positive immediate skin tests in cockroach-allergic asthmatic patients, suggesting that GST from cockroach is a clinically relevant allergen[75]. Regarding helminth-induced IgE cross-reactivity, Santiago *et al*. showed that the GST from the filarial worm *Wuchereria bancrofti* (WbGST) and cockroach GST (Bla g 5) were 30% identical at the amino acid with marked similarity in the N-terminal region[76]. Interestingly, mice infected with *Heligmosomoides bakeri*; a parasite that contains a GST that is 32% identical to Bla g 5, developed immediate hypersensitivity reaction in the skin to cockroach GST (Bla g 5), suggesting that some parasite-induced cross-reactivity may induce *in vivo* reactivity to the cross-reactive allergen in a common allergen source like house dust mite[76].

Paramyosin is another allergen family from invertebrate muscle that are targeted in IgE responses against helminths[77]. A study among patients reporting symptoms of allergy and another group of *Ascaris*-infected subject in the Philippines showed evidence of cross-reactivity between paramyosin from mite (*B. tropicalis*) and paramyosin from *A. lumbricoides*[78]. This study observed that IgE to mite extract among allergic patients can be inhibited, up to 92%, by *Ascaris* antigen while mite extract could inhibit up to 54% of *Ascaris*-sIgE among *Ascaris*-

infected subjects. Of note, IgE responses to the recombinant form of the paramyosin *Blomia* allergen (Blo t 11) were seen in 80% of allergic patients and 46% of *Ascaris*-infected subjects[78].

In general, IgE cross-reactivity between helminth antigens and allergens demonstrates a limited to diagnostic value of examining IgE responses to whole allergen extracts in helminth-endemic populations. Establishing the molecular basis of cross-reactivity between helminths and common allergen sources is essential to evaluate whether sensitization to the latter is true primary sensitization or cross-reactivity induced by helminths.

COMPONENT-RESOLVED DIAGNOSIS (CRD) IN ALLERGY DIAGNOSIS

For the past few decades, *in vitro* allergy diagnostics has been largely based on the detection of specific IgE to whole extracts comprised of allergenic and non-allergenic components. However, this approach has been problematic since the allergenic content of whole extracts is often difficult to standardize and also the specific allergic reaction inducing components in whole allergen extracts can be hard to identify. Such issues in *in vitro* allergy diagnostics led to the development of component-resolved diagnostics (CRD) in which purified natural or recombinant allergens are used to detect IgE sensitization to individual allergen molecules[79,80].

The use of molecular techniques and recombinant DNA technology has allowed the sequencing, synthesizing and cloning of allergenic proteins leading to the production of recombinant allergens for CRD[81]. Recombinant allergens can be generated as defined molecules with consistent quality and without biological variation[82].

The molecular biological techniques underlying CRD were initially employed for the determination of the primary structures and molecular identities of allergens[83]. The sequence analysis of allergens allowed the identification of structurally related allergens and also revealed how closely linked cross-reactive molecules may not be differentiated by the immune system[83]. CRD involves the use of specific marker allergens to identify primary sensitization towards a particular allergen source and to discriminate it from sensitization to CCD or to homologous allergens as a result of cross-reactivity[84]. CRD can therefore facilitate the differentiation between clinically important and less relevant specific IgEs[85].

In terms of the application of CRD to research questions in helminth-endemic populations, the report by Amoah *et al.* on peanut allergy among Ghanaian school children found that in study subjects with elevated IgE to whole peanut allergen, responses to recombinant major peanut allergens (rAra h 1, rAra h 2 and rAra h 3) were generally undetectable or very low (<1 kU_A/L)[65]. In addition, among Brazilian children living in a helminth-endemic urban area, Carvalho *et al.* evaluated the use of IgE responses to *B. tropicalis* allergens (rBlo t 5 and rBlo t 21) in improving the specificity of determining mite allergy in this population[86]. The study showed that the assays using these recombinant marker allergens for primary *B. tropicalis* sensitization, exhibited no IgE cross-reactivity with *A. lumbricoides* antigens and therefore conferred higher specificity in detecting primary mite IgE sensitization than crude mite extract.

1

SCOPE OF THE THESIS

In recent years, much research from the developed world has looked at risk as well as protective factors for childhood allergy. The sharp rise in atopic disorders in the developed world within a relatively short period of time indicates that environmental factors, in addition to the already known genetic pre-disposition, play an important role in development and control of these diseases. However, there are no conclusive answers on which are the most important environmental changes that underlie the increase in atopic disorders. Interestingly, investigators are shifting their attention to the developing world for clues on how changes in the environment, influenced by socio-economic status (SES), affect the development of atopic disorders. One of these environmental factors is helminth infections. Indonesia is one of the low-to-middle income countries which is in epidemiological transition with increasing level of urbanization accompanied by changing disease patterns; where helminth infections are highly prevalent in rural areas but in urban centers there is a rise in atopic disorders. Therefore, this thesis set out to address the following four questions in Indonesia:

- Are the risk factors of atopic disorders different between children belonging to low or high socio-economic status living in the same urban area?
- What are the risk factors for atopic disorders in children living in rural and semi urban areas where helminth infections are highly prevalent?
- What is the effect of long term anthelmintic treatment on atopic disorders?
- What is the profile of allergen specific IgE antibodies in children from helminth-endemic areas?

In **Chapter 2** and **Chapter 4**, we show how environmental factors and socio-economic status effect atopic disorders in schoolchildren in different areas of a developing country.

Chapter 3 provides an overview of the study population used in the following chapter 4, 5 and 6. It has been published as a study protocol paper.

Chapter 5 provides the result of a household-based-cluster-randomized, double-blind placebo-controlled anthelminthic trial on allergy.

In **Chapter 6**, we look at the characteristics of allergen specific IgE antibodies in order to better understand the molecular basis of IgE recognition of allergens in a helminth-endemic area.

Chapter 7 summarizes our findings and provides directions for future research to understand the link between helminth infections, socio-economic status and atopic disorders.

REFERENCES

1 Pawankar R, Canonica GW, et al. Introduction and Executive Summary: Allergic Diseases as a Global Public Health Issue. In: Pawankar R, Canonica GW, Holgate S.T., LOckey RF, editors. World Allergy Organization (WAO) White Book on Allergy. 2011:11-20.

2 Weinmayr G, Weiland SK, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2005;176:565-74.

3 Yemaneberhan H, Bekele Z, et al. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. Lancet 1997;350:85-90.

4 Nicolaou N, Siddique N, et al. Allergic disease in urban and rural populations: increasing prevalence with increasing urbanization. Allergy 2005;60:1357-60.

5 Addo Yobo EO, Custovic A, et al. Exercise induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. Thorax 1997;52:161-5.

6 Hamid F, Wiria AE, et al. Risk factors associated with the development of atopic sensitization in Indonesia. PLoS One 2013;8:e67064.

7 Perzanowski MS, Ng'ang'a LW, et al. Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. J Pediatr 2002;140:582-8.

8 Kopel LS, Phipatanakul W, et al. Social disadvantage and asthma control in children. Paediatr Respir Rev 2014;15:256-62.

9 Braveman P. Health disparities and health equity: concepts and measurement. Annu Rev Public Health 2006;27:167-94.

10 Braveman PA, Cubbin C, et al. Socioeconomic status in health research: one size does not fit all. JAMA 2005;294:2879-88.

11 Kozyrskyj AL, Kendall GE, et al. Association between socioeconomic status and the development of asthma: analyses of income trajectories. Am J Public Health 2010;100:540-6.

12 Lindbaek M, Wefring KW, et al. Socioeconomical conditions as risk factors for bronchial asthma in children aged 4-5 yrs. Eur Respir J 2003;21:105-8.

13 Almqvist C, Pershagen G, et al. Low socioeconomic status as a risk factor for asthma, rhinitis and sensitization at 4 years in a birth cohort. Clin Exp Allergy 2005;35:612-8.

14 Gong T, Lundholm C, et al. Parental socioeconomic status, childhood asthma and medication use--a population-based study. PLoS One 2014;9:e106579.

15 Victorino CC, Gauthier AH. The social determinants of child health: variations across health outcomes - a population-based cross-sectional analysis. BMC Pediatr 2009;9:53.

16 Poyser MA, Nelson H, et al. Socioeconomic deprivation and asthma prevalence and severity in young adolescents. Eur Respir J 2002;19:892-8.

17 Hancox RJ, Milne BJ, et al. Relationship between socioeconomic status and asthma: a longitudinal cohort study. Thorax 2004;59:376-80.

18 Mitchell EA, Stewart AW, et al. Socioeconomic status in childhood asthma. Int J Epidemiol 1989;18:888-90.

19 Burke W, Fesinmeyer M, et al. Family history as a predictor of asthma risk. Am J Prev Med 2003;24:160-9.

20 von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. J Allergy Clin Immunol 2002;109:S525-S532.

21 Bethony J, Brooker S, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521-32.

22 Yazdanbakhsh M, van den Biggelaar A, et al. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001;22:372-7.

23 Feary J, Britton J, et al. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66:569-78.

24 Obihara CC, Beyers N, et al. Respiratory atopic disease, Ascaris-immunoglobulin E and tuberculin testing in urban South African children. Clin Exp Allergy 2006;36:640-8.

25 Palmer LJ, Celedon JC, et al. Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002;165:1489-93.

26 Basomba A, Sastre A, et al. Standardization of the prick test. A comparative study of three methods. Allergy 1985;40:395-9.

27 Wide L, Bennich H, et al. Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 1967;2:1105-7.

28 Priftanji A, Strachan D, et al. Asthma and allergy in Albania and the UK. Lancet 2001;358:1426-7.

29 Cooper PJ, Chico ME, et al. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. Am J Respir Crit Care Med 2003;168:313-7.

30 Dagoye D, Bekele Z, et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am J Respir Crit Care Med 2003;167:1369-73.

31 Supali T, Djuardi Y, et al. Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. Int Arch Allergy Immunol 2010;153:388-94.

32 van den Biggelaar AH, Lopuhaa C, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. Int Arch Allergy Immunol 2001;126:231-8.

33 Cooper PJ, Chico ME, et al. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004;34:845-52.

34 Flohr C, Tuyen LN, et al. Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: A cross-sectional study. J Allergy Clin Immunol 2006;118:1305-11.

35 Cooper PJ, Chico ME, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 2003;111:995-1000.

36 Araujo MI, Lopes AA, et al. Inverse association between skin response to aeroallergens and Schistosoma mansoni infection. Int Arch Allergy Immunol 2000;123:145-8.

37 Obeng BB, Amoah AS, et al. Schistosome infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian schoolchildren. Clin Exp Allergy 2014;44:965-75.

38 Scrivener S, Yemaneberhan H, et al. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet 2001;358:1493-9.

39 Ponte EV, Lima F, et al. Skin test reactivity and Der p-induced interleukin 10 production in patients with asthma or rhinitis infected with Ascaris. Ann Allergy Asthma Immunol 2006;96:713-8.

40 Amberbir A, Medhin G, et al. Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clin Exp Allergy 2011;41:1422-30.

41 Cooper PJ, Chico ME, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006;367:1598-603.

42 Flohr C, Tuyen LN, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2010;40:131-42.

43 Schabussova I, Wiedermann U. Allergy and worms: let's bring back old friends? Wien Med Wochenschr 2014;164:382-91.

44 Smits HH, Hammad H, et al. Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol 2007;120:932-40.

45 Smits HH, Everts B, et al. Chronic helminth infections protect against allergic diseases by active regulatory processes. Curr Allergy Asthma Rep 2010;10:3-12.

46 Larson D, Hubner MP, et al. Chronic helminth infection reduces basophil responsiveness in an IL-10-dependent manner. J Immunol 2012;188:4188-99.

47 Royer B, Varadaradjalou S, et al. Inhibition of IgE-induced activation of human mast cells by IL-10. Clin Exp Allergy 2001;31:694-704.

48 Macaubas C, Sly PD, et al. Regulation of T-helper cell responses to inhalant allergen during early childhood. Clin Exp Allergy 1999;29:1223-31.

49 van den Biggelaar AH, van Ree R, et al. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. Lancet 2000;356:1723-7.

50 Godfrey RC. Asthma and IgE levels in rural and urban communities of The Gambia. Clin Allergy 1975;5:201-7.

51 Editorial:IgE, parasites, and allergy. Lancet 1976;1:894-5.

52 Lynch NR, Hagel IA, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol 1998;101:217-21.

53 Mitre E, Norwood S, et al. Saturation of immunoglobulin E (IgE) binding sites by polyclonal IgE does not explain the protective effect of helminth infections against atopy. Infect Immun 2005;73:4106-11.

54 MacGlashan DW, Jr., Bochner BS, et al. Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic

patients with anti-IgE antibody. J Immunol 1997;158:1438-45.

55 Yazdanbakhsh M, Kremsner PG, et al. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.

56 Amoah AS, Obeng BB, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol 2013;132:639-47.

57 Larson D, Cooper PJ, et al. Helminth infection is associated with decreased basophil responsiveness in human beings. J Allergy Clin Immunol 2012;130:270-2.

58 Aalberse RC, Akkerdaas J, et al. Cross-reactivity of IgE antibodies to allergens. Allergy 2001;56:478-90.

59 Acevedo N, Caraballo L. IgE cross-reactivity between Ascaris lumbricoides and mite allergens: possible influences on allergic sensitization and asthma. Parasite Immunol 2011;33:309-21.

60 Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol 2007;142:99-115.

61 van Ree R, Cabanes-Macheteau M, et al. Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J Biol Chem 2000;275:11451-8.

62 Aalberse RC, Koshte V, et al. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981;68:356-64.

63 van der Veen MJ, van Ree R, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 1997;100:327-34.

64 Mari A, Ooievaar-de HP, et al. Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. Allergy 2008;63:891-6.

65 Amoah AS, Obeng BB, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol 2013;132:639-47.

66 Amoah AS, Boakye DA, et al. Parasitic worms and allergies in childhood: insights from population studies 2008-2013. Pediatr Allergy Immunol 2014;25:208-17.

67 Arruda LK, Santos AB. Immunologic responses to common antigens in helminthic infections and allergic disease. Curr Opin Allergy Clin Immunol 2005;5:399-402.

68 Sereda MJ, Hartmann S, et al. Helminths and allergy: the example of tropomyosin. Trends Parasitol 2008;24:272-8.

69 Jenkins JA, Breiteneder H, et al. Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy Clin Immunol 2007;120:1399-405.

70 Santiago HC, Bennuru S, et al. Structural and immunologic cross-reactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis. J Allergy Clin Immunol 2011;127:479-86.

71 Santos AB, Rocha GM, et al. Cross-reactive IgE antibody responses to tropomyosins from Ascaris lumbricoides and cockroach. J Allergy Clin Immunol 2008;121:1040-6.

72 Acevedo N, Sanchez J, et al. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009;64:1635-43.

73 Sheehan D, Meade G, et al. Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 2001 Nov 15;360(Pt 1):1-16.

74 Acevedo N, Mohr J, et al. Proteomic and immunochemical characterization of glutathione transferase as a new allergen of the nematode Ascaris lumbricoides. PLoS One 2013;8:e78353.

75 Arruda LK, Vailes LD, et al. Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (Blattella germanica). J Biol Chem 1997;272:20907-12.

76 Santiago HC, LeeVan E, et al. Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. J Allergy Clin Immunol 2012;130:248-56.

77 Fitzsimmons CM, Falcone FH, et al. Helminth Allergens, Parasite-Specific IgE, and Its Protective Role in Human Immunity. Front Immunol 2014;5:61.

78 Valmonte GR, Cauyan GA, et al. IgE cross-reactivity between house dust mite allergens and Ascaris lumbricoides antigens. Asia Pac Allergy 2012;2:35-44.

79 Treudler R, Simon JC. Overview of component resolved diagnostics. Curr Allergy Asthma Rep 2013;13:110-7.

80 Valenta R, Lidholm J, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999;29:896-904.

81 Gadisseur R, Chapelle JP, et al. A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP(c) 250 with the ImmunoCAP(c) ISAC. Clin Chem Lab Med 2011;49:277-80.

82 Valenta R, Niederberger V. Recombinant allergens for immunotherapy. J Allergy Clin Immunol 2007;119:826-30.

83 Valenta R, Lidholm J, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999;29:896-904.

84 De Knop KJ, Bridts CH, et al. Component-resolved allergy diagnosis by microarray: potential, pitfalls, and prospects. Adv Clin Chem 2010;50:87-101.

85 Treudler R, Simon JC. Overview of component resolved diagnostics. Curr Allergy Asthma Rep 2013;13:110-7.

86 Carvalho KA, de Melo-Neto OP, et al. Blomia tropicalis Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. BMC Immunol 2013;14:11.

2

ALLERGIC DISORDERS AND SOCIO-ECONOMIC STATUS: A STUDY OF SCHOOL CHILDREN IN AN URBAN AREA OF MAKASSAR, INDONESIA

Firdaus Hamid[1,2], Sitti Wahyuni[3], Astrid van Leeuwen[4], Ronald van Ree[5], Maria Yazdanbakhsh[2], Erliyani Sartono[2]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
4. Department of Immunopathology, Sanquin Research, Amsterdam, The Netherlands
5. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Clinical & Experimental Allergy 2015;45:1226-36

ABSTRACT

Background In urban centres of developing countries, there is great variation in socio-economic status (SES) and lifestyle; however, little information is available on allergic disorders in groups with high- or low-SES within the same urban area.

Objective To determine the prevalence of allergic disorders and investigate risk factors related to them among high- and low-SES schoolchildren in Makassar, the capital city of South Sulawesi, Indonesia.

Method This cross-sectional study was performed in 623 children originating from high- (N = 349) and low-SES (N = 274) schools. Information on reported allergic symptoms and potential factors associated with allergic disorders was obtained by questionnaire. Specific IgE and skin prick test (SPT) reactivity were determined against aeroallergens [*Dermatophagoides pteronyssinus* [HDM] and cockroach]. Total IgE and helminth infections were also assessed.

Result The prevalence of SPT to any aeroallergens was significantly higher in high-SES than in low-SES school (25% vs. 8%, $P < 0.001$, respectively). However, specific IgE against cockroach and total IgE were significantly lower in high- than in low-SES children. Allergic symptoms were reported more often in low- compared to high-SES children. Specific IgE to aeroallergens significantly increased the risk of SPT positivity to the same aeroallergen in the high-, but not in the low-SES children. In the high- but not in low-SES, there was a significant positive association between SPT to HDM and wheeze. Similarly, cockroach skin reactivity and elevated BMI increased the risk of eczema in the high-SES children only.

Conclusion and Clinical Relevance Skin prick test positivity is more frequent in high-SES, whereas IgE and allergic symptoms are higher in low-SES children. Specific IgE is a risk factor for being SPT-positive, and SPT positivity is a risk factor for allergic symptoms but only in children of high- and not low-SES school. Therefore, the socio-economic status of a child might affect the diagnosis of allergic disease in a developing country.

INTRODUCTION

It has long been known that allergic diseases cluster within families and this is likely to be due to genetic predisposition. However, environmental factors may modulate expression of allergic disorders. A higher prevalence of allergies in developed countries compared to developing ones[1], and the great differences in prevalence between urban and rural populations particularly in developing[2-6] but also in developed countries[7-9] has clearly shown how important the influence of environmental factors is on the expression of allergic disorders.

The worldwide International Study of Asthma and Allergies in Childhood (ISAAC) has reported that Indonesia is one of the countries with low prevalence of allergy in the world[1]. However, this study reported data from only one center in Java. A study which was conducted in 10 centers in India reported a large variation in the prevalence of asthma in the different centers (ranging from 3% to 17%), indicating that the information on allergic disorders in Indonesia reported by the published ISAAC study may not be representative of the whole country.

Several factors related to western lifestyle such as increase in exposure to outdoor pollutants[10], increased indoor allergen load[11], altered diet[12,13] and changes in exposure to infection/microbial products[14,15] have been hypothesized to explain the increase in allergic disorders. Socio-economic status (SES) also can affect allergic disorders, as studied in affluent countries[16-18]. However, there are not many studies addressing the pattern of allergic disorders within an urban center in a developing country where large differences in SES and life style are seen.

To investigate this, we initiated a study in two schools with different socio-economic backgrounds (high- and low-SES school) in an urban area of Makassar, South Sulawesi, to measure the prevalence of atopy and reported clinical allergy. Data on several factors such as parental education, parental occupation, the presence of smokers in house, pets in house, nutritional status and helminth infections were collected to determine how these factors influence the allergic phenotype.

METHODS

Study area and design

The study was conducted in two elementary schools in Makassar, the capital city of South Sulawesi, Indonesia. Data were collected between October and December 2005. One school was attended by children from families with low-SES (SD Cambaya), and was located at the periphery of the city, near a port. The children from this school lived in the surrounding area and came from families with low education level who mostly worked as fishermen, menial laborers, or some that were skilled, but working in low ranking jobs. The high-SES school (SD Mangkura) was located in the city centre, about 7 km from the low-SES school. The houses of these children were spread in different parts of the city and had good sanitary facilities. The children went to school by private vehicles or by a school bus.

A month prior to the start of the study, the parents of children in both schools from third to sixth grades were sent a letter informing them of the study and asking them to

sign a letter if they agreed for their child to participate in the study. Only children who returned the signed letters were included in the study. The study was approved by the ethical committees of Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (ref:0147/H4.8.4.5.31/PP36-KOMETIK/2005). In total 274 children from the low-SES and 349 from high-SES were included in the study (Figure S1).

Questionnaires

Reported clinical symptoms of allergy were obtained by questionnaire. Clinical symptoms of asthma, allergic rhinitis and atopic dermatitis (eczema) in the previous 12 months were assessed using a modified ISAAC questionnaire (supplementary questionnaire 1-3), which had been translated into Bahasa Indonesia. Children were identified to have asthma symptoms (wheeze) if wheezing was reported in the past 12 months by parents or guardian. Rhinitis was defined by a positive response to the questions, 'In the past 12 months, has your child had a problem with sneezing, or a runny or a blocked nose and has this nose problem been accompanied by itchy watery eyes?'. Eczema in the past 12 months was determined by a positive response to the questions, 'Has your child had one or more skin problems accompanied by an itchy rash in the previous 12 months?'.

An additional questionnaire was applied to obtain data on parental education, parental occupation, the number of siblings and pet contact inside the house as well as smokers in the house. Parental occupation was classified into 2 groups of low- and high-skill jobs. Educational levels were categorized as: 'low' for illiterate, elementary school or high school and 'high' for academic/university and above. The questionnaire was administered to the parents or guardians of children.

Skin prick testing

Skin prick test (SPT) was performed if children were free from anti-histamine, anti-asthmatic or corticosteroid drugs for at least 7 days prior to the testing. SPT reactivity to aeroallergens was tested with extract of *Dermatophagoides pteronyssinus* (house dust mite (HDM); HAL Allergen BV, Leiden, The Netherlands) and *Blattella germanica* (cockroach; Lofarma, Milan, Italy). Histamin chloride (10 mg/ml) was used as the positive control and allergen diluents as the negative control. SPT was done on the volar side of the child's lower arm, using separate skin prick test. The results for each child were measured after 15 minutes. Skin prick reactivity was determined to be positive if the longest diameter plus the diameter perpendicular of wheal size divided by two was at least 3 mm. Body height and weight were also measured.

Specific and total IgE

Serum level of mite- and cockroach-IgE was determined by radio allergosorbent test (RAST) as described previously[19]. Briefly, 50 µl serum was incubated overnight with 1.5 mg of Sepharose-coupled allergen in a final volume of 300 µl PBS, 3% BSA, 0.1% Tween-20. After

washing away non-bound serum components, radiolabelled sheep antibodies (Sanquin, Amsterdam, The Netherlands) directed to human IgE, were added. After overnight incubation and washing, bound radioactivity was measured. The outcomes were expressed as % binding. To convert these values into IU/ml, the result were plotted to non-linear regression curve of chimeric monoclonal IgE antibody dilution series against the major house dust mite allergen, Der p 2 and Sepharose-coupled mite extracts.

The levels of total IgE were measured by ELISA in The Netherlands as described previously[20,21]. The results were expressed as International Units (IU/ml).

Parasitological examination

The children were asked to fill a pot carefully using wooden spatula without water or urine contamination. The time of stool passed had to be recorded and the stool had to be stored in a cool area if it stayed overnight in the house before delivery to school. Only stools that arrived in the laboratory not more than 12 hours after passage were examined. The eggs from intestinal helminth such as *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm were quantified using the Kato Katz methods[22].

Statistical analysis

The collected data were analyzed using IBM Statistical Package for Social Sciences (IBM Corp., Armonk, New York, USA) version 20. We investigated potential factors for allergic disorders separately for each school. Age-standardized z-scores of body mass index (z-BMI) were calculated according to WHO references values[23]. Descriptive data were expressed as means (± standard deviations), frequency (percentage of collected data) and geometric means [95% confidence intervals (CI)]. Prevalence rates were calculated and compared for different schools using Pearson chi-square tests, while comparisons of continuous data were analyzed by using Student *t*-tests. Specific IgE (s-IgE) and total IgE were normalized by log-transformation to obtain normally distributed data. Logistic regression was used to analyze the associations between the potential factors and development of SPT and reported clinical symptoms of allergy in the past 12 months. Linear regression was used for analysis of continuous outcomes which provided estimated regression coefficients (β) and their corresponding 95% CI. In multivariate analysis, we included age and sex as *a priori* confounders, as well as other variables that were significant in univariate analyses. All statistical tests were considered significant at $P < 0.05$.

RESULTS

Characteristics of study participants

Among 917 children invited to the study, 71 (7.7%) refusals came from high-SES whereas 223 (24.3%) were from low-SES (Figure S1). One of the reasons could have been illiteracy, but we have no data on this. Thus, a total of 349 children from the high-SES and 274 children from

the low-SES school were included in the study (Figure S1). Children were slightly younger in the high-SES compared to the low-SES school (mean age 9.05 vs. 9.92 years; $P < 0.001$), whereas sex distribution was similar in both schools (Table 1). This slight age difference did not affect the results, as repeating the analysis after matching the study population for age, revealed identical results for the outcomes reported in this study. Occupation and education of the parents were homogeneous within the schools but very different between schools: in the high-SES school, 98% and 65% of parents had a high-skill occupation and high education, respectively; whereas in the low-SES school, 84% (230/274) of parents had a low-skill occupation and almost all parents had low education (97%, 255/264) (Table 1).

Almost all children (90%) from low-SES school were infected with at least one species of helminth compared to 22% in high-SES school (Table 1). The most common helminth infections were *T. trichiura* (87% in low-SES and 19% in high-SES) and *A. lumbricoides* (low-SES: 77%, high-SES: 6%). The prevalence of hookworm infection was very low (9 of 611, 1.5%); therefore, hookworm infection was excluded from further analysis.

Prevalence of reported symptoms, skin prick test and IgE

The prevalence of reported wheeze in the previous 12 months was lower in the high-SES (7.5%) compared to the low-SES school (12.9%) as were the prevalence of reported symptoms of eczema (9.9% in high-SES school and 18.2% in low-SES school) and allergic rhinitis (26.6% vs. 41.3%, $P = 0.001$, respectively) in the past 12 months (Table 1).

For analysis of skin reactivity to aeroallergens, we included only children with a positive skin test (≥ 3 mm) to histamine (Table 1). There were no differences in age and sex distribution between the histamine-negative population (N = 133 in high-SES and N = 77 in low-SES) and the histamine-positive population (high-SES: 216 children, high-SES: 197 children). The prevalence of positive SPT was higher in the high-SES school compared to low-SES school; any aeroallergen (25% vs. 8.1%, $P < 0.001$, respectively), HDM (15.7% vs 3%, $P < 0.001$, respectively) and cockroach (16.2% vs 6.1%, $P = 0.001$, respectively). In contrast, the levels of sIgE to cockroach as well as total IgE were significantly lower in the high-SES than in the low-SES. There were no differences in the levels of HDM sIgE between the two schools (Table 1).

Potential risk factors associated with reported clinical symptoms of allergy in the past 12 months

In the high-SES school, reported wheeze in the previous 12 months was significantly associated with SPT reactivity to HDM [odds ratio (OR), 3.18; 95% CI, 1.17–8.62; $P = 0.023$]. Skin reactivity to cockroach (OR, 3.40; 95% CI, 1.39–8.29; P = 0.007) and z-BMI (OR, 1.31; 95% CI, 1.02–1.69; P = 0.032) were positively associated with an increased risk for reported clinical symptoms of eczema in the past 12 months (Table 2a). However, we found no association between rhinitis and potential risk factors measured in the high-SES school (data not shown).

In the low-SES school, none of the exposures assessed were significantly associated with risk for reported clinical symptoms of allergy (Table 2b).

Table 1. Characteristics of population and allergic disorders in high- and low-socio-economic status (SES) schools

	High-SES		Low-SES		
	N	**Result**	**N**	**Result**	***P*-value**
Age years (mean, SD)	349	9.05 ± 1.22	274	9.92 ± 1.62	**<0.001**
Sex (N, %)					
Male	162	46.4	145	52.9	0.11
Female	187	53.6	129	47.1	
Parental job (N, %)					
Low skill	8	2.3	230	83.9	**<0.001**
High skill	341	97.7	44	16.1	
Parental education (N, %)					
Low	117	35.0	255	96.6	**<0.001**
High	217	65.0	9	3.4	
Smoker inside the house (N, %)					
No	162	48.4	76	28.8	**<0.001**
Yes	173	51.6	188	71.2	
Pet inside house (N, %)					
No	294	84.2	232	84.7	0.88
Yes	55	15.8	42	15.3	
z-BMI (mean, SD)	349	-0.08 ± 1.39	274	-0.80 ± 1.24	**<0.001**
Number of siblings (N, %)					
< 3	214	61.3	99	36.1	**<0.001**
3+	135	38.7	175	63.9	
Helminth infection (N, n%)					
Any intestinal helminth	340	76 (22.4)	271	245 (90.4)	**<0.001**
Ascaris lumbricoides	340	20 (5.9)	271	208 (76.8)	**<0.001**
Trichuris trichiura	340	65 (19.1)	271	236 (87.1)	**<0.001**
Clinical symptoms of allergy in the past 12 months (N, n%)					
Wheeze	335	25 (7.5)	264	34 (12.9)	**0.027**
Rhinitis	335	89 (26.6)	264	109 (41.3)	**<0.001**
Eczema	335	33 (9.9)	264	48 (18.2)	**0.003**
Skin prick test reactivity (N, n%)					
Any skin prick test reactivity	216	54 (25.0)	197	16 (8.1)	**<0.001**
Dermatophagoides pteronyssinus	216	34 (15.7)	197	6 (3.0)	**<0.001**
Blattella germanica	216	35 (16.2)	197	12 (6.1)	**0.001**
Specific IgE and Total IgE (geometric mean, 95% CI)					
House dust mite[#] (IU/ml)	272	0.27 (0.21-0.36)	243	0.24 (0.20-0.30)	0.55
B. germanica (IU/ml)	272	0.08 (0.07-0.10)	242	0.31 (0.27-0.36)	**<0.001**
Total IgE (IU/ml)	272	1267.6 (1024.8-1568.0)	240	12925.9 (10834.6-15420.9)	**<0.001**

The number of positives (n) of the total population examined (N). The statistically significant results are given in bold. SD: standard deviation. z-BMI: z score of Body Mass Index. CI: Confidence intervals. [#]IgE to *D. pteronyssinus*.

Table 2. Association between potential risk factors and clinical symptoms of allergic diseases in (a) high- and (b) low-SES schools^

		Wheeze		Eczema	
	N	***n* (%)**	**OR [95% CI]**	***n* (%)**	**OR [95% CI]**
(a)					
Parental job					
Low	8	0		0	
High	327	25 (7.6)	-	33 (10.1)	-
Parental education					
Low	117	5 (4.3)	reference	10 (8.5)	reference
High	217	20 (9.2)	2.27 [0.83-6.23]	23 (10.6)	1.27 [0.58-2.76]
Smoker inside the house					
No	162	10 (6.2)	reference	18 (11.1)	reference
Yes	173	15 (8.7)	1.44 [0.63-3.31]	15 (8.7)	0.76 [0.37-1.56]
Pet inside house					
Low	282	24 (8.5)	reference	28 (9.9)	reference
High	53	1 (1.9)	0.21 [0.03-1.56]	5 (9.4)	0.94 [0.35-2.57]
z-BMI∞	335	-0.10 ± 1.37§	0.86 [0.63-1.17]	-0.10 ± 1.37§	**1.31 [1.02-1.69]***
Number of siblings					
< 3	205	14 (6.8)	reference	19 (9.3)	reference
3+	130	11 (8.5)	1.26 [0.55-2.87]	14 (10.8)	1.18 [0.57-2.45]
Any intestinal helminth					
Negative	254	21 (8.3)	reference	28 (11.0)	reference
Positive	76	4 (5.3)	0.62 [0.20-1.85]	5 (6.6)	0.57 [0.21-1.53]
A. lumbricoides					
Negative	310	24 (7.7)	reference	31 (10.0)	reference
Positive	20	1 (5.0)	0.63 [0.08-4.89]	2 (10.0)	1.00 [0.22-4.51]
T. trichiura					
Negative	265	21 (7.9)	reference	29 (10.9)	reference
Positive	65	4 (6.2)	0.76 [0.25-2.30]	4 (6.2)	0.53 [0.18-1.58]
HDM SPT					
Negative	301	19 (6.0)	reference	29 (9.6)	reference
Positive	34	6 (17.6)	**3.18 [1.17-8.62]***	4 (11.8)	1.25 [0.41-3.80]
Cockroach SPT					
Negative	301	22 (7.3)	reference	25 (8.3)	reference
Positive	34	3 (8.8)	1.23 [0.35-4.34]	8 (23.5)	**3.40 [1.39-8.29]****

Table 2. *Continued*

		Wheeze		Eczema	
	N	***n* (%)**	**OR [95% CI]**	***n* (%)**	**OR [95% CI]**
(b)					
Parental job					
Low	222	34 (15.3)		45 (20.3)	reference
High	42	0	-	3 (7.1)	0.30 [0.09-1.02]
Parental education					
Low	255	34 (13.3)		47 (18.4)	reference
High	9	0	-	1 (11.1)	0.55 [0.07-4.53]
Smoker inside the house					
No	76	10 (13.2)	reference	14 (18.4)	reference
Yes	188	24 (12.8)	0.97 [0.44-2.13]	34 (18.1)	0.98 [0.49-1.95]
Pet inside house					
Low	223	28 (12.6)	reference	41 (18.4)	reference
High	41	6 (14.6)	1.19 [0.46-3.09]	7 (17.1)	0.91 [0.38-2.21]
z-BMI∞	264	-0.79 ± 1.25[§]	0.88 [0.66-1.17]	-0.79 ± 1.25[§]	1.17 [0.90-1.51]
Number of siblings					
< 3	93	13 (14.0)	reference	15 (16.1)	reference
3+	171	21 (12.3)	1.26 [0.55-2.87]	33 (19.3)	1.24 [0.64-2.43]
Any intestinal helminth					
Negative	21	2 (9.5)	reference	4 (19.0)	reference
Positive	241	32 (13.3)	1.45 [0.32-6.54]	44 (18.3)	0.95 [0.30-2.96]
A. lumbricoides					
Negative	58	4 (6.9)	reference	10 (17.2)	reference
Positive	204	30 (14.7)	2.33 [0.78-6.90]	38 (18.6)	1.10 [0.51-2.37]
T. trichiura					
Negative	30	3 (10.0)	reference	6 (20.0)	reference
Positive	232	31 (13.4)	1.39 [0.40-4.85]	42 (18.1)	0.88 [0.34-2.30]
HDM SPT					
Negative	258	32 (12.4)	reference	47 (18.2)	reference
Positive	6	2 (33.3)	3.53 [0.62-20.06]	1 (16.7)	0.90 [0.10-7.87]
Cockroach SPT					
Negative	253	31 (12.3)	reference	44 (17.4)	reference
Positive	11	3 (27.3)	2.69 [0.68-10.66]	4 (36.4)	2.71 [0.76-9.67]

^association based on univariate logistic model. ∞Increase in risk of clinical symptoms of allergy for an increasing of each unitary in the tested variable. [§]Mean and standard deviation. The number of positives (n) of the total population examined (N). OR: Odds ratio, CI: Confidence intervals. The statistically significant results are given in bold. $^{*}P < 0.05$, $^{**}P < 0.01$

Potential risk factors associated with skin prick test reactivity

In high-SES school, skin reactivity to HDM was positively associated with high levels of sIgE to HDM (OR, 6.03; 95% CI, 3.34–10.88; $P < 0.001$), and skin reactivity to cockroach was positively associated with high levels of sIgE to cockroach (OR, 5.64; 95% CI, 2.18–14.63; $P < 0.001$) (Table 3a).

In the low-SES school, higher z-BMI was associated with SPT reactivity to cockroach. However, no significant association was found between skin reactivity and sIgE (Table 3b).

Potential risk factors associated with total and allergen-specific IgE

None of the measured potential risk factors were associated with total IgE or sIgE to aeroallergens in the high-SES school (Table S1a).

In the low-SES school, having parents with high-skill occupation ($\beta = -0.31$; $P = 0.014$) or high education ($\beta = -0.63$; $P = 0.023$) was associated with lower levels of sIgE to HDM. Levels of sIgE to cockroach as well as total IgE ($\beta = 0.21$; $P = 0.012$: $\beta = 0.23$; $P = 0.021$, respectively) were significantly higher in children with *T. trichiura* infections (Table S1b).

Multivariate analysis

In high-SES school, skin reactivity to HDM was an independent predictor of reported wheeze in the past 12 months (adjusted OR, 3.21; 95% CI, 1.17–8.78; $P = 0.023$) while eczema was independently associated with positive skin reactivity to cockroach as well as high z-BMI. Analysis of skin reactivity adjusted for confounding factors revealed that skin reactivity to HDM remained positively associated with sIgE to HDM (adjusted OR, 6.19; 95% CI, 3.40–11.28; $P < 0.001$) while skin reactivity to cockroach remained positively associated with sIgE to cockroach (adjusted OR, 5.68; 95% CI, 2.13–15.18; $P < 0.001$) (Table 4).

In low-SES school, multivariate analysis revealed that high z-BMI was still associated with cockroach SPT reactivity (adjusted OR, 1.74; 95% CI, 1.02–2.96; $P = 0.041$; Table 4) and having parents with high-skill occupation was still associated with having low levels of sIgE to HDM (adjusted $\beta = -0.28$; $P = 0.030$). Following adjustment with age and sex, infection with *T. trichiura* remained positively associated with high levels of sIgE to cockroach (adjusted $\beta = 0.22$; $P = 0.011$) as well as total IgE (adjusted $\beta = 0.23$; $P = 0.022$).

DISCUSSION

This study has investigated allergic disorders in high-and low-SES school children living in the same urban centre of a developing country, namely Makassar, Indonesia. We observed the prevalence of skin prick test reactivity to aeroallergen was higher in high-SES compared to the low-SES school. Conversely, the prevalence of reported allergic symptoms, IgE to cockroach as well as total IgE were higher in low-SES compared to high-SES school children. In the high-SES school, high sIgE to aeroallergens increased the risk of skin reactivity to the

Table 3. Association between potential risk factors of allergy and skin reactivity in (a) high- (b) low-SES school^

		Any SPT		HDM SPT		Cockroach SPT	
	N	n	OR [95% CI)	n	OR [95% CI)	n	OR [95% CI)
(a)							
Parental job							
Low	3	0		0		0	
High	213	54 (25.4)	-	34 (16.0)	-	35 (16.4)	-
Parental education							
Low	68	14 (20.6)	reference	11 (16.2)	reference	10 (14.7)	reference
High	140	39 (27.9)	1.49 [0.74-2.98]	23 (16.4)	1.02 [0.46-2.23]	24 (17.1)	1.20 [0.54-2.68]
Smoker inside the house							
No	105	31 (29.5)	reference	20 (19.0)	reference	17 (16.2)	reference
Yes	104	22 (21.2)	0.64 [0.34-1.20]	14 (13.5)	0.66 [0.31-1.39]	17 (16.3)	1.01 [0.49-2.11]
Pet inside house							
No	184	50 (27.2)	reference	32 (17.4)	reference	32 (17.4)	reference
Yes	32	4 (12.5)	0.38 [0.13-1.15]	2 (6.3)	0.32 [0.07-1.39]	3 (9.4)	0.49 [0.14-1.71]
z-BMI∞	216	-0.09 ± 1.40§	1.10 [0.88-1.37]	-0.09 ± 1.40§	1.10 [0.85-1.43]	-0.09 ± 1.40§	0.93 [0.71-1.21]
Number of siblings							
< 3	123	27 (22.0)	reference	17 (13.8)	reference	18 (14.6)	reference
3+	93	27 (29.0)	1.45 [0.78-2.70]	17 (18.3)	1.39 [0.67-2.91]	17 (18.3)	1.30 [0.63-2.70]
Any intestinal helminth							
Negative	165	46 (27.9)	reference	28 (17.0)	reference	28 (17.0)	reference
Positive	44	8 (18.2)	0.57 [0.25-1.33]	6 (13.6)	0.77 [0.30-2.00]	7 (15.9)	0.93 [0.37-2.29]

Table 3. *Continued*

		Any SPT		HDM SPT		Cockroach SPT	
	N	n	OR [95% CI)	n	OR [95% CI)	n	OR [95% CI)
Ascaris lumbricoides							
Negative	201	53 (26.4)	reference	33 (16.4)	reference	34 (16.9)	reference
Positive	8	1 (12.5)	0.40 [0.05-3.32]	1 (12.5)	0.73 [0.09-6.11]	1 (12.5)	0.70 [0.08-5.89]
Trichuris trichiura							
Negative	170	46 (27.1)	reference	28 (16.5)	reference	28 (16.5)	reference
Positive	39	8 (20.5)	0.70 [0.30-1.62]	6 (15.4)	0.92 [0.35-2.41]	7 (17.9)	1.11 [0.45-2.76]
Specific IgE to HDM	164		-		**6.03 [3.34-10.88]*** **		-
Specific IgE to cockroach	164		-		-		**5.64 [2.18-14.63]*** **
(b)							
Parental job							
Low	163	13 (8.0)	reference	5 (3.1)	reference	9 (5.5)	reference
High	34	3 (8.8)	1.12 [0.30-4.15]	1 (2.9)	0.96 [0.11-8.47]	3 (8.8)	1.66 [0.42-6.47]
Parental education							
Low	183	14 (7.7)	reference	5 (2.7)	reference	10 (5.5)	reference
High	6	1 (16.7)	2.41 [0.26-22.12]	1 (16.7)	7.12 [0.70-72.72]	1 (16.7)	3.46 [0.37-32.49]
Smoker inside the house							
No	51	5 (9.8)	reference	2 (3.9)	reference	5 (9.8)	reference
Yes	138	10 (7.2)	0.72 [0.23-2.21]	4 (2.9)	0.73 [0.13-4.12]	6 (4.3)	0.42 [0.12-1.44]

Table 3. *Continued*

		Any SPT		HDM SPT		Cockroach SPT	
	N	n	OR [95% CI)	n	OR [95% CI)	n	OR [95% CI)
Pet inside house							
No	170	15 (8.8)	reference	5 (2.9)	reference	12 (7.1)	
Yes	27	1 (3.7)	0.40 [0.05-3.14]	1 (3.7)	1.27 [0.14-11.30]	0	-
z-BMI∞	197	-0.80 ± 1.26[§]	1.55 [0.99-2.43]	-0.80 ± 1.26[§]	1.14 [0.59-2.21]	-0.80 ± 1.26[§]	**1.73 [1.03-2.92]***
Number of siblings							
< 3	69	8 (11.6)	reference	2 (2.9)	reference	6 (8.7)	reference
3+	128	8 (6.2)	0.51 [0.18-1.42]	4 (3.1)	1.08 [0.19-6.05]	6 (4.7)	0.52 [0.16-1.67]
Any intestinal helminth							
Negative	17	1 (5.9)	reference	1 (5.9)	reference	1 (5.9)	reference
Positive	178	15 (8.4)	1.47 [0.18-11.88]	5 (2.8)	0.46 [0.05-4.20]	11 (6.2)	1.05 [0.13-8.70]
Ascaris lumbricoides							
Negative	43	1 (2.3)	reference	1 (2.3)	reference	1 (2.3)	reference
Positive	152	15 (9.9)	4.60 [0.59-35.85]	5 (3.3)	1.43 [0.16-12.57]	11 (7.2)	3.28 [0.41-26.12]
Trichuris trichiura							
Negative	23	1 (4.3)	reference	1 (4.3)	reference	1 (4.3)	reference
Positive	172	15 (8.7)	2.10 [0.26-16.70]	5 (2.9)	0.66 [0.07-5.90]	11 (6.4)	1.50 [0.18-12.21]
Specific IgE to HDM	171		-		2.70 [0.61-11.91]		-
Specific IgE to cockroach	171		-		-		3.13 [0.66-14.92]

^association based on univariate logistic model. ∞Increase in risk of skin prick reactivity for an increasing of each unitary in the tested variable. [§]Mean and standard deviation. The number of positives (n) of the total population examined (N). OR: Odds ratio, CI: Confidence intervals. The statistically significant results are given in bold. ***$P < 0.001$. *$P < 0.05$.

Table 4. Multivariate models for association between potential risk factors and clinical symptoms of allergy or skin reactivity in (a) high- and (b) low-SES schools^

	Clinical symptom of allergy in the past 12 months		Skin prick test reactivity	
	HDM adj. OR [95% CI]	**Cockroach adj. OR [95% CI]**	**adj. OR [95% CI]**	**adj. OR [95% CI]**
(a)				
z-BMI∞		1.38 [1.06-1.78]*		
SPT HDM [reference:negative]	3.21 [1.17-8.78]*			
SPT cockroach [reference:negative]		3.81 [1.53-9.52]**		
Specific IgE to HDM			6.19 [3.40-11.28]***	
Specific IgE to cockroach				5.68 [2.13-15.18]***
(b)				
z-BMI∞				1.74 [1.02-2.96]*

^Multivariate model adjusted with age and sex. ∞Increase in risk of clinical symptoms of allergy or skin prick reactivity for an increasing of each unitary in the tested variable. OR: Odds ratio. CI: Confidence intervals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

same aeroallergens, and moreover, skin reactivity to HDM increased the risk of reported wheeze. In contrast to the findings among the high-SES children, in the low-SES school, sIgE did not significantly increase the risk of being SPT-positive and SPT was not a significant risk factor for clinical symptoms of allergy. Studies in children among 22 countries worldwide found large variation in the prevalence of allergic symptoms and atopic sensitization among populations and also reported that the association between atopic sensitization and clinical symptoms of asthma increased with economic development[7]. The latter would be in line with our observation that in high-SES sensitization is linked to clinical symptoms whereas in low-SES, this is not the case.

Most studies on the association between BMI and allergic disorders in children are in high-income countries[24–26] while little is known on this association in children from low-to-middle income countries. Among high-SES school children in this study, we also found that skin reactivity to cockroach and BMI were positively associated with the increased risk of eczema. There are to our knowledge, no published reports on the association between eczema and cockroach sensitization while a similar trend for association between eczema and BMI has been reported by Yao *et al.*[27].

The fact that the prevalence of wheeze, allergic rhinitis and atopic eczema symptoms was lower in high-SES school children was opposite to the finding from a previous study conducted in children attending 30 schools in socio-economically diverse areas of Cape Town, South Africa, which reported that the prevalence of asthma, recent wheeze and allergic rhinitis increased from lowest to highest SES[28,29]. One of the possibilities to consider is that certain viral infections,

which might be associated with allergy-like symptoms and difficult to differentiate from real allergy by parents, were more prevalent in the low-SES children of the current study[30,31].

High parental education and occupation, which are part of the indicators of high-SES, have been reported to be associated with atopy[32,33]. Here, we found no association between skin prick test reactivity or reported clinical symptoms of allergy and parental education nor with parental occupation, most likely due to homogeneity of these variables in each of high- and low-SES schools in our setting.

We could not find any association between allergic outcome measured and exposure to tobacco smoke or having pets at home which is similar to the findings is a study of rural and urban of Ecuador[3]. Although studies in Germany showed that being born and raised on a livestock farm protected against atopy and allergic symptoms[34]; however, significant heterogeneity effects have also been reported across Europe[35].

Both helminth parasites and allergens are associated with Th2 immune responses characterized by the increased production of Th2 cytokines and with specific as well as polyclonal IgE[36]. In this study, total IgE levels were 10 times higher in the low-SES than in the high-SES school. In low-SES school, the levels of total IgE were significantly higher in children infected with *T. trichiura* where the prevalence of this infection was 87.1%. In the high-SES school where the prevalence of *T. trichiura* infection was much lower, infection with this parasite was not significantly associated with increased levels of total IgE, suggesting that levels of total IgE in low-SES school are likely to be the consequence of higher transmission of *T. trichiura* and other helminth such as *A. lumbricoides* which was also prevalent in low-SES school. In line with this, a study by Blackwell *et al.*[37] showed that in rural Ecuador and Bolivia, total IgE increased with increasing helminth positivity and decreased in parallel with reduction of helminth infestation[38].

Interestingly, the levels of sIgE to cockroach were higher in children infected with *T. trichuria* in the low-SES school. Additionally, in the same school, we found that having parents with high-skill occupation significantly reduced sIgE levels to HDM, which might be because high-skill occupation means less exposure to helminths. It is also possible that IgE antibodies generated to helminth antigens might cross-react with allergens[39,40] as was shown by a recent study among Ghanaian children, which demonstrated that high levels of IgE to peanut were strongly associated with helminth infection[41].

In multivariate analysis of data from the high-SES school, we found that the levels of sIgE to aeroallergens are strongly associated with the skin reactivity to the same aeroallergens. This is consistent with several studies which found a good agreement between SPT and sIgE in developed[42–44] and in an urban area of developing country[45]. However, no significant association was observed in the low-SES which is in line with our previous study conducted in a rural area of Indonesia where a dissociation between sIgE levels to aeroallergens and skin prick test to the same allergens was found[2]. These data show that despite living in the same city, socio-economic differences might result in different association between sIgE and SPT reactivity.

The strength of this study is the relatively large number of children examined that lived in the same area. Weaknesses were cross-sectional design and the use of questionnaires

to obtain information on clinical symptoms of allergic disease. The assessment of clinical symptoms of allergy by questionnaire could under or overestimate the real cases of allergic diseases. The other limitation of our current study was that the participant response rate particularly in low-SES was lower than in high-SES school, probably due to illiteracy but we have no data on this. In addition, in the low-SES school, the numbers of children with positive SPT were lower and therefore our studies of associations involving SPT might be underpowered. Confounding factors included in the study were limited; therefore, it is possible that we missed important potential confounding factors. The presence of helminth infection was determined by single Kato–Katz, which might miss light infections.

In conclusion, there are large differences between children from high- and low-SES schools in an urban area of Indonesia with respect to allergic disorders and factors that influence allergic outcomes. There is high IgE in low-SES but low SPT, while reported symptoms of allergy are higher in low-SES children. Our data also provide evidence that specific IgE is a risk factor for being SPT-positive and SPT positivity is a risk factor for allergic symptoms but only in children of high-SES and not low-SES school. Therefore, one needs to consider SES when testing for allergic disorders in cities in developing countries.

REFERENCES

1 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998;351:1225-32.

2 Hamid F, Wiria AE, et al. Risk factors associated with the development of atopic sensitization in Indonesia. PLoS ONE 2013;8:e67064.

3 Cooper PJ, Chico ME, et al. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004;34:845–52.

4 Davey G, Venn A, et al. Wheeze, allergic sensitization and geohelminth infection in Butajira, Ethiopia. Clin Exp Allergy 2005;35:301–7.

5 Nyan OA, Walraven GE, et al. Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. Clin Exp Allergy 2001;31:1672–8.

6 Perzanowski MS, Ng'ang'a LW, et al. Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. J Pediatr 2002;140:582–8.

7 Weinmayr G, Weiland SK, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2007;176:565–74.

8 Wong GW, Chow CM. Childhood asthma epidemiology: insights from comparative studies of rural and urban populations. Pediatr Pulmonol 2008;43:107–16.

9 Priftanji A, Strachan D, Burr M et al. Asthma and allergy in Albania and the UK. Lancet 2001;358:1426–7.

10 Kim KH, Jahan SA, et al. A review on human health perspective of air pollution with respect to allergies and asthma. Environ Int 2013;59:41–52.

11 Morgan WJ, Crain EF, et al. Results of a home-based environmental intervention among urban children with asthma. N Engl J Med 2004;351:1068–80.

12 Chen R, Hu Z, et al. Eating more vegetables might explain reduced asthma symptoms. BMJ 2004;328:1380.

13 Hooper R, Calvert J, et al. Urban/rural differences in diet and atopy in South Africa. Allergy 2008;63:425–31.

14 Yazdanbakhsh M, Kremsner PG, et al. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490–4.

15 von Mutius E. Infection: friend or foe in the development of atopy and asthma? The epidemiological evidence. Eur Respir J 2001;18:872–81.

16 Basagana X, Sunyer J, et al. Socioeconomic status and asthma prevalence in young adults:

the European Community Respiratory Health Survey. Am J Epidemiol 2004;160:178–88.

17 Jansson SA, Protudjer JL, et al. Socioeconomic evaluation of well-characterized allergy to staple foods in adults. Allergy 2014;69:1241–7.

18 Thakur N, Martin M, et al. Socioeconomic status and asthma control in African American youth in SAGE II. J Asthma 2014;51:720–8.

19 Aalberse RC, Koshte V, et al. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981;68:356–64.

20 Hamid F, Wiria AE, et al. A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2011;11:83.

21 Wiria AE, Prasetyani MA, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2010;10:77.

22 Katz N, Chaves A, et al. A simple device for quantitative stool thick-smear technique in Schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo 1972;14:397–400.

23 WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. Geneva: World Health Organization 2006:312.

24 Tanaka K, Miyake Y, et al. U-shaped association between body mass index and the prevalence of wheeze and asthma, but not eczema or rhinoconjunctivitis: the ryukyus child health study. J Asthma 2011;48:804–10.

25 Fitzpatrick S, Joks R, et al. Obesity is associated with increased asthma severity and exacerbations, and increased serum immunoglobulin E in inner-city adults. Clin Exp Allergy 2012;42:747–59.

26 Bruske I, Flexeder C, et al.. Body mass index and the incidence of asthma in children. Curr Opin Allergy Clin Immunol 2014;14:155–60.

27 Yao TC, Ou LS, et al. Associations of age, gender, and BMI with prevalence of allergic diseases in children: PATCH study. J Asthma 2011;48:503–10.

28 Mercer MJ, Joubert G, et al. Socioeconomic status and prevalence of allergic rhinitis and atopic eczema symptoms in young adolescents. Pediatr Allergy Immunol 2004;15:234–41.

29 Poyser MA, Nelson H, et al. Socioeconomic deprivation and asthma prevalence and severity in young adolescents. Eur Respir J 2002;19:892–8.

30 Cashat-Cruz M, Morales-Aguirre JJ, et al. Respiratory tract infections in children in developing countries. Semin Pediatr Infect Dis 2005;16:84–92.

31 Heymann PW, Carper HT, et al. Viral infections in relation to age, atopy, and season of admission among children hospitalized for wheezing. J Allergy Clin Immunol 2004;114:239–47.

32 Forastiere F, Agabiti N, et al. Socioeconomic status, number of siblings, and respiratory infections in early life as determinants of atopy in children. Epidemiology 1997;8:566–70.

33 Cooper PJ, Chico ME, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 2003;111:995–1000.

34 Von Ehrenstein OS, von Mutius E, et al. Reduced risk of hay fever and asthma among children of farmers. Clin Exp Allergy 2000;30:187–93.

35 Ege MJ, Frei R, et al. Not all farming environments protect against the development of asthma and wheeze in children. J Allergy Clin Immunol 2007;119:1140–7.

36 Yazdanbakhsh M, van den Biggelaar A, et al. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001;22:372–7.

37 Blackwell AD, Gurven MD, et al. Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. PLoS Negl Trop Dis 2011;5:e1218.

38 Flohrs K, Bruske I, et al. Temporal changes in total serum immunoglobulin E levels in East German children and the effect of potential predictors. Int Arch Allergy Immunol 2012;158:27–34.

39 van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002;129:189–97.

40 Acevedo N, Sanchez J, et al. IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009;64:1635–43.

41 Amoah AS, Obeng BB, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children

possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol 2013;132:639–47.

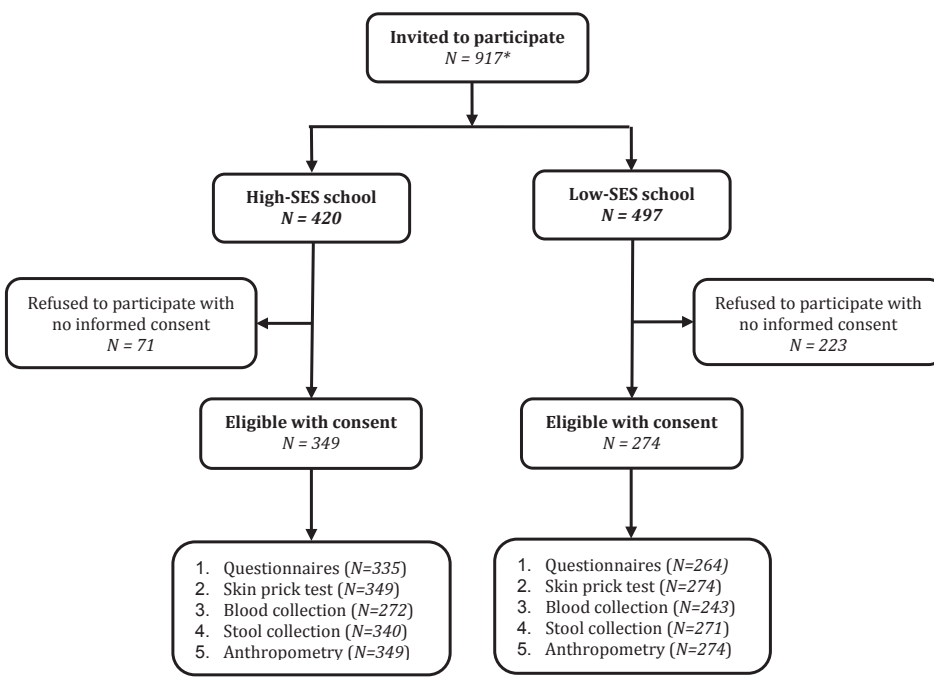
42 Ollert M, Weissenbacher S, et al. Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing. Clin Chem 2005;51:1241–9.

43 Onell A, Hjalle L, et al. Exploring the temporal development of childhood IgE profiles to allergen components. Clin Transl Allergy 2012;2:24.

44 Ro AD, Saunes M, et al. Agreement of specific IgE and skin prick test in an unselected cohort of two-year-old children. Eur J Pediatr 2012;171:479–84.

45 Obeng BB, Amoah AS, et al. Food allergy in Ghanaian schoolchildren: data on sensitization and reported food allergy. Int Arch Allergy Immunol 2011;155:63–73.

SUPPLEMENTARY FIGURE



* children from third to sixth grades of elementary school
N, number
SES, socio-economic status

Figure S1. Flow chart of the study. *The flow chart showing participation at each school and the measurements collected.*

SUPPLEMENTARY TABLE

Table S1. Association between potential risk factors of allergy and specific or total IgE in (a) high- and (b) low-SES schools^

		IgE to HDM#		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β (95% CI)	geometric mean [95% CI]	β (95% CI)	N	geometric mean [95% CI]	β (95% CI)
(a)								
Parental job								
Low	6	0.47 [0.08-2.73]	reference	0.18 [0.07-0.50]	reference	6	538.0 [51.4-5635.3]	reference
High	261	0.27 [0.21-0.36]	-0.25 (-1.05-0.55)	0.08 [0.06-0.09]	-0.38 (-0.99-0.23)	261	1351.5 [1087.6-1679.3]	0.39 (-0.24-1.02)
Parental education								
Low	96	0.22 [0.14-0.37]	reference	0.09 [0.06-0.12]	reference	96	1177.7 [829.1-1672.8]	reference
High	171	0.31 [0.22-0.43]	0.14 (-0.10-0.39)	0.07 [0.06-0.10]	-0.09 (-0.28-0.10)	171	1413.6 [1072.5-1863.1]	0.08 (-0.12-0.28)
Smoker inside the house								
No	133	0.29 [0.19-0.45]	reference	0.07 [0.05-0.09]	reference	132	1160.2 [865.1-1556.0]	reference
Yes	134	0.26 [0.18-0.36]	-0.05 (-0.29-0.18)	0.09 [0.07-0.12]	0.12 (-0.06-0.31)	135	1505.9 [1094.6-2071.8]	0.11 (-0.07-0.30)
Pet inside house								
No	226	0.28 [0.20-0.38]	reference	0.07 [0.06-0.09]	reference	226	1390.6 [1091.9-1771.0]	reference
Yes	41	0.27 [0.14-0.50]	-0.01 (-0.33-0.32)	0.10 [0.07-0.15]	0.14 (-0.11-0.39)	41	1008.9 [634.3-1604.8]	-0.13 (-0.39-0.13)
z-BMI	272	-0.16 ± 1.34$	0.08 (-0.01-0.17)	-0.15 ± 1.34$	0.02 (-0.05-0.09)	272	-0.15 ± 1.35$	0.00 (-0.07-0.07)
Number of siblings								
< 3	165	0.24 [0.17-0.33]	reference	0.07 [0.05-0.09]	reference	164	1153.9 [868.9-1532.3]	reference
3+	107	0.33 [0.20-0.52]	0.13 (-0.11-0.37)	0.10 [0.07-0.13]	0.15 (-0.03-0.33)	108	1550.9 [1119.9-2147.9]	0.13 (-0.06-0.32)

Table S1 Continued

		IgE to HDM#		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β (95% CI)	geometric mean [95% CI]	β (95% CI)	N	geometric mean [95% CI]	β (95% CI)
Any intestinal helminth								
Negative	205	0.29 [0.21-0.39]	reference	0.07 [0.06-0.10]	reference	205	1338.8 [1044.0-1716.8]	reference
Positive	62	0.24 [0.13-0.45]	-0.06 (-0.34-0.22)	0.09 [0.06-0.14]	0.09 (-0.13-0.30)	62	1275.4 [814.5-1997.0]	-0.01 (-0.23-0.21)
Ascaris lumbricoides								
Negative	250	0.28 [0.21-0.37]	reference	0.08 [0.06-0.10]	reference	250	1365.4 [1086.9-1715.4]	reference
Positive	17	0.25 [0.10-0.59]	-0.04 (-0.53-0.44)	0.06 [0.02-0.19]	-0.13 (-0.50-0.24)	17	839.3 [498.2-1414.0]	-0.20 (-0.59-0.18)
Trichuris trichiura								
Negative	215	0.29 [0.21-0.39]	reference	0.07 [0.06-0.09]	reference	215	1312.0 [1032.7-1666.8]	reference
Positive	52	0.23 [0.11-0.46]	-0.09 (-0.39-0.21)	0.09 [0.06-0.14]	0.10 (-0.13-0.33)	52	1373.6 [817.3-2308.4]	0.03 (-0.21-0.27)
(b)								
Parental job								
Low	198	0.27 [0.22-0.34]	reference	0.33 [0.28-0.38]	reference	194	12675.9 [10678.9-15046.5]	reference
High	37	0.14 [0.07-0.28]	**-0.31 (-0.56--0.06)***	0.26 [0.18-0.39]	-0.12 (-0.28-0.04)	37	9850.5 [6339.9-15305.0]	-0.14 (-0.32-0.04)
Parental education								
Low	228	0.25 [0.21-0.31]	reference	0.32 [0.28-0.37]	reference	224	12087.3 [10279.4-14213.2]	
High	7	0.06 [0.00-1.00]	**-0.63 (-1.18--0.09)***	0.20 [0.06-0.63]	-0.20 (-0.55-0.14)	7	15306.6 [4706.0-49785.6]	0.10 (-0.30-0.51)
Smoker inside the house								
No	68	0.23 [0.15-0.36]	reference	0.34 [0.26-0.45]	reference	68	12507.6 [9120.2-17153.2]	
Yes	167	0.25 [0.19-0.32]	0.02 (-0.18-0.23)	0.31 [0.26-0.36]	-0.04 (-0.17-0.09)	163	12037.7 [9997.2-14494.5]	-0.02 (-0.17-0.14)

Table S1 Continued

		IgE to HDM[#]		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β (95% CI)	geometric mean [95% CI]	β (95% CI)	N	geometric mean [95% CI]	β (95% CI)
Pet inside house								
No	199	0.24 [0.19-0.31]	reference	0.31 [0.26-0.35]	reference	196	11647.7 [9732.1-13940.4]	reference
Yes	36	0.25 [0.17-0.38]	0.01 (-0.25-0.26)	0.39 [0.28-0.54]	0.08 (-0.08-0.24)	35	15593.4 [11418.2-21295.5]	0.11 (-0.08-0.30)
z-BMI	243	-0.80 ± 1.24[§]	0.06 (-0.02-0.13)	-0.80 ± 1.24[§]	0.04 (-0.01-0.09)	240	-0.80 ± 1.24[§]	0.02 (-0.04-0.07)
Number of siblings								
< 3	86	0.28 [0.19-0.40]	reference	0.31 [0.25-0.39]	reference	84	12430.6 [9623.6-16056.3]	reference
3+	157	0.23 [0.17-0.30]	-0.09 (-0.28-0.11)	0.31 [0.26-0.37]	0.00 (-0.12-0.12)	156	11463.0 [9392.3-13990.2]	-0.04 (-0.18-0.11)
Any intestinal helminth								
Negative	20	0.27 [0.14-0.51]	reference	0.23 [0.14-0.37]	reference	19	8009.1 [4576.8-14015.1]	reference
Positive	215	0.24 [0.19-0.30]	-0.01 (-0.31-0.29)	0.33 [0.28-0.38]	0.16 (-0.03-0.35)	212	12639.7 [10699.0-14932.4]	0.23 (0.00-0.45)*
Ascaris lumbricoides								
Negative	52	0.29 [0.20-0.41]	reference	0.30 [0.23-0.40]	reference	51	11839.7 [8225.2-17042.7]	reference
Positive	183	0.23 [0.18-0.30]	-0.07 (-0.28-0.15)	0.32 [0.28-0.38]	0.05 (-0.09-0.18)	180	12270.6 [10264.0-14669.5]	0.04 (-0.12-0.20)
Trichuris trichiura								
Negative	28	0.20 [0.09-0.43]	reference	0.21 [0.14-0.31]	reference	27	7874.6 [5019.0-12354.8]	reference
Positive	207	0.25 [0.20-0.31]	0.11 (-0.15-0.38)	0.34 [0.29-0.39]	0.21 (0.05-0.38)*	204	12896.7 [10879.3-15288.2]	0.23 (0.04-0.43)*

^association based on univariate linear model. [§]Mean and standard deviation. The total population examined [N). [#]IgE to *Dermatophagoides pteronyssinus* [HDM). β (beta): estimate regression coefficients. CI: Confidence intervals. The statistically significant results are given in bold. *$P < 0.05$.

SUPPLEMENTARY QUESTIONNAIRES

The International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires

2

1. Core questionnaire for wheezing and asthma (all questions are about problems which occur when this child DOES NOT have cold or the flu)

No	Question	Answer
1	Have you ever had wheezing or whistling in the chest at any time in the past?	[ ] Yes [ ] No If no skip to Q6
2	Have you had wheezing or whistling in the chest in the last 12 months?	[ ] Yes [ ] No If no skip to Q6
3	How many attacks of wheezing have you had in the last 12 months?	[ ] None [ ] 4-12 [ ] 1-3 [ ] > 12
4	In the last 12 months, how often, on average, has your sleep been disturbed due to wheezing?	[ ] Never woken with wheezing [ ] Less than one night per week [ ] One or more nights per week
5	In the last 12 months, has wheezing ever been severe enough to limit your speech to only one or two words at a time between breaths?	[ ] Yes [ ] No
6	Have you ever had asthma? Diagnosed by a doctor	[ ] Yes [ ] No
7	In the last 12 months, has your chest sounded wheezy during or after exercise?	[ ] Yes [ ] No
8	In the last 12 months, have you had a dry cough at night, apart from a cough associated with a cold or chest infection?	[ ] Yes [ ] No
9	If yes for question number 6, what is the name of medicine that Doctor gave	
10	Has any member of your family ever had asthma?	[ ] Yes [ ] No [ ] No idea
11	If you answered "yes" to question 10, indicate relationship to you (*tick all that apply*)	[ ] Father [ ] Mother [ ] Brother or Sister [ ] Mother's family _____ [ ] Father's family_____

2. Core questionnaire for allergic rhinitis (all questions are about problems which occur when this child DOES NOT have a cold or the flu)

No	Question	Answer
1	Have you ever had a problem with sneezing or a runny or blocked nose (nose problem) without cold or the flu?	[ ] Yes [ ] No If no skip to Q6
2	In the past 12 months, have you had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu?	[ ] Yes [ ] No If no skip to Q6
3	In the past 12 months, has this nose problem been accompanied by itchy-watery eyes?	[ ] Yes [ ] No
4	In which of the past 12 months did this nose problem occur? (Please tick any which apply)	[ ] Jan [ ] Mei [ ] Sep [ ] Feb [ ] Jun [ ] Oct [ ] Mar [ ] Jul [ ] Nov [ ] Apr [ ] Aug [ ] Dec [ ] Rainy season [ ] Dry season [ ] Anytime [ ] No idea
5	In the past 12 months, how much did this nose problem interfere with your daily activities?	[ ] Not at all [ ] A Moderate [ ] A little [ ] A lot
6	Have you had a doctor diagnosed rhinitis allergy / hay fever?	[ ] Yes [ ] No
7	If yes for question number 6, what is the name of medicine that Doctor gave	
8	Has any member of your family ever had rhinitis allergy / hay fever?	[ ] Yes [ ] No
9	If you answered "yes" to question 8, indicate relationship to you (*tick all that apply*)	[ ] Father [ ] Mother [ ] Brother or Sister [ ] Mother's family _____ [ ] Father's family_____

2

3. Core questionnaire for eczema (show the pictures to the subject)

No	Question	Answer
1	Have you ever had an itchy rash which was coming and going for at least six months?	[ ] Yes, picture number_____ [ ] No If no skip to Q6
2	Have you had this itchy rash at any time in the last 12 months?	[ ] Yes [ ] No If no skip to Q6
3	Has this itchy rash at any time affected any of the following places: The folds of the elbows, behind the knees, in front of ankles, under the buttocks or around the neck, ears or eyes?	[ ] Yes [ ] No
4	Has this rash cleared completely at any time during the past 12 months?	[ ] Yes [ ] No
5	In the last 12 months, how often, on average, have you been kept awake by this itchy rash?	[ ] Never in the past 12 months [ ] Less than one night per week [ ] One or more nights per week
6	Have you had Doctor diagnosed dermatitis allergy/eczema?	[ ] Yes [ ] No
7	If yes for question number 6, what is the name of medicine that Doctor gave	----------
8	Has any member of your family ever had had allergy eczema?	[ ] Yes [ ] No
9	If you answered "yes" to question 8, indicate relationship to you (*tick all that apply*)	[ ] Father [ ] Mother [ ] Brother or Sister [ ] Mother's family _____ [ ] Father's family_____

3.1 Modification in Indonesian version of ISSAC Questionnaire for eczema

No	Before	Questions of eczema	Answer
1	Modify from Q1	Have you ever had skin problem that occur more than once in the same location?	1. Yes 2. No, **You don't need to continue**
2	Modify from Q6	Have your skin problem been diagnosed by doctor/nurse as dermatitis allergy?	1. Yes 2. No
Additional	SHOW ECZEMA PICTURE		
3	Modify from Q1	Have you ever had itchy skin problem that look like in the picture?	1. Yes 2. No
4	Modify from Q3	Which area	Fossa cubiti Fossa poplitea Fossa Axiller Inguinale Dorsum pedis Dorsum palmar Another area ……
5	Q2	In the last 12 month have you ever had the symptoms?	1. Yes 2. No, **You don't need to continue**.
6	Q4	In the last 12 month, did your skin disorders completely disappear?	1. Yes 2. No
7	Modify from Q5	In the last 12 month how many times you weak at night due to itchy of your skin disorders	1. 1-3 x/ year 2. 4-12 x/year 3. >12x/year

A LONGITUDINAL STUDY OF ALLERGY AND INTESTINAL HELMINTH INFECTIONS IN SEMI URBAN AND RURAL AREAS OF FLORES, INDONESIA (IMMUNOSPIN STUDY)

Firdaus Hamid[1,2], Aprilianto E Wiria[2,3], Linda J Wammes[2], Maria MM Kaisar[2,3], Bertrand Lell[4], Iwan Ariawan[5], Hae Won Uh[6], Heri Wibowo[3], Yenny Djuardi[3], Sitti Wahyuni[7], Robert Schot[8], Jaco J Verweij[2], Ronald van Ree[9], Linda May[2], Erliyani Sartono[2], Maria Yazdanbakhsh[2], Taniawati Supali[3]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
4. Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon; Department of Parasitology, Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany
5. Department of Biostatistics, School of Public Health, University of Indonesia, Jakarta, Indonesia
6. Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands
7. Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
8. Department of Pulmonology, Leiden University Medical Center, Leiden. The Netherlands
9. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

BMC Infectious Diseases 2011;11:83

ABSTRACT

Background The prevalence of asthma and atopic disease has been reported to be low in low income countries, however helminth infections are likely to be high among these communities. The question of whether helminth infections play a role in allergic diseases can best be addressed by intervention studies. None of the studies so far have been based on a large scale placebo-controlled trial.

Method/Design This study was designed to assess how intestinal helminth infections can influence the immune response and atopic and allergic disorders in children in Indonesia. The relations between allergic outcomes and infection and lifestyle factors will be addressed. This study was set up among school-age children in semi urban and rural areas, located in Ende District of Flores Island, Indonesia. A randomized placebo-controlled anthelmintic treatment trial to elucidate the impact of helminth infections on the prevalence of skin prick test (SPT) reactivity and symptoms of allergic diseases will be performed. The children living in these semi-urban and rural areas will be assessed for SPT to allergens before and after 1 and 2 years of treatment as the primary outcome of the study; the secondary outcome is symptoms (asthma and atopic dermatitis); while the tertiary outcome is immune responses (both antibody levels to allergens and cellular immune responses).

Discussion The study will provide information on the influence of helminth infections and anthelmintic treatment on immune response, atopy and allergic disorders.

Trial registration Current Controlled Trials ISRCTN: ISRCTN83830814

INTRODUCTION

Helminth infections are highly prevalent worldwide, with more than two billion people chronically infected by soil transmitted helminths such as *Ascaris lumbricoides*, *Trichuris trichiura* and/or hookworms (*Necator americanus* or *Ancylostoma duodenale*)[1]. These enteric infections affect populations living in subtropical and tropical regions of low-middle income countries, where access to hygiene, sanitation and source of clear water is limited[2]. The immune responses mounted to helminth infections is characterized by T-helper type 2 (Th2), which are thought to be protective[3]. However, there is also evidence that these parasites might enhance their own survival by modulating the immune responses of their host by inducing regulatory responses that dampen activity of effector cells[4]. Whether all different helminths are equally potent in inducing regulatory responses is not yet fully studied.

Allergens, like helminth antigens[5,6] are potent inducers of Th2 responses[7] and it is known that allergic diseases including asthma, eczema and rhinitis are associated with Th2 inflammation[8]. However, in contrast to helminth infections the Th2 associated allergic diseases, which are the most common cause of chronic disease of childhood in high income countries, appear to be less common in low income countries[9]. Thus, despite the close parallels between immune responses that characterize helminth infections and allergic diseases, namely increased levels of Immunoglobulin (Ig)-E, tissue eosinophilia and mastocytosis along with T cells that preferentially secrete Th2 cytokines interleukin (IL)-4, IL-5 and IL-13[8,10-12], the clinical outcome with respect to immediate hypersensitivity and inflammation is clearly not the same[13]. Indeed, often it has been reported that these diseases, appear to segregate geographically[14] and several studies have reported a negative association between the presence of helminth infections and allergic disorders[15-18]. In experimental animal models, several parasitic helminths have been shown to prevent the development of eosinophilic airway inflammation and hyperresponsiveness[19-21].

Mechanistically, a number of immune responses have been proposed to account for the negative association between helminths and allergies[22]. The observations that chronic helminth infections are associated with higher suppressive responses, such as IL-10[23] and regulatory T cells[24,25] have led to the proposal that a strong regulatory network induced by helminths might prevent the downstream effector phase of Th2 responses, preventing excess inflammation. Moreover, the possibility that in the presence of helminth infections, IgE antibodies generated are of lower affinity and therefore can not lead to mast cell degranulation has also been put forward[22].

Given that a number of studies have on the other hand reported either no[26,27] or a positive[28,29] association between helminths and allergies, it is very likely that, apart from the source and chronicity of infection other factors such as exposure to non-helminth infections, and/or lifestyle play an important role in the development of allergies. The change from traditional to a more "modern" lifestyle which encompasses not only reduced exposure to micro-organisms and parasites but also an altered diet, in addition to changes in degree of manual labour or inhalation of pollutants is clearly associated with changing

disease patterns[2]. It is important to study and delineate the mechanisms that may protect from the development of allergic diseases. It is becoming clear that the prevalence of allergic diseases is increasing in low to middle income countries[30] particularly in urban centers which often show higher prevalence of these diseases compared to rural areas[14,31,32]. It is therefore important to use this window of opportunity to identify risk and protective factors in cross sectional as well as longitudinal studies.

A study that would include both helminth infections and life style factors with respect to the development of allergies has been planned in Indonesia. The question of whether helminth infections play a role in allergic diseases can best be addressed by intervention studies. So far, one intervention study has suggested that anthelmintic treatment might increase the incidence of atopy reactivity[33], which is in contrast to a large scale study where one year after treatment of intestinal helminths no changes were recorded in allergic disorders[34]. None of the studies have been based on a large scale placebo controlled trial. Although there clearly are ethical issues with such a design, the ethics committee of University of Indonesia, has granted permission for a placebo controlled trial providing that the community gets extensive medical care and excludes those with intense infections. In addition to helminth infections, the study of how other factors may contribute to the development of allergies is best achieved by longitudinal comparison of different areas along a rural-urban gradient. Numerous studies have analyzed the difference in the prevalence of allergic disorders in a rural to urban gradient[35] but none so far has done so in a longitudinal manner with the exception of one study in Ghana[31].

The ImmunoSPIN allergy project http://www.immunospin.org website[36] has been initiated with this aim. This study is a randomized placebo-controlled anthelmintic treatment trial to elucidate the impact of helminth infections on the prevalence of atopy and allergic diseases. In this study the prevalence of IgE, skin prick test positivity and symptoms of allergic diseases such as asthma and atopic dermatitis in school-age children will be assessed in semi-urban and rural area in Flores, Indonesia. The ImmunoSPIN allergy project will establish the risk and protective factors and will include immune response measurements in order to understand the immunological mechanisms that are behind risk and protective factors in allergy development.

METHODS/DESIGN

The study area

For this study semi-urban (Nangapanda) and rural (Anaranda) sites located in Ende District of Flores Island, Indonesia were selected. Nangapanda is a sub-district situated in a coastal area with a population of approximately 22,000 (Figure 1c). Nangapanda is divided into 17 villages of which those located near the community health centre (Puskesmas), Ndeturea, Ndorurea 1, and Ndorurea together with a population of 4650[36], were included in the study. Local income in this area is based on fishing and farming while some engage in jobs at government officers with a few in the private sector.

Anaranda is a village in sub-district of Welamosa and is located 80 km north from Nangapanda with a population of approximately 1,600 (Figure 1d). The majority of income is generated by farming. The infrastructure is poor with no paved roads which makes the village isolated and with little access to amenities as were available in Nangapanda, such as electricity (which is provided 12 hours per day), fuel, natural water source (not processed water) and shops.

Preliminary surveys in 2005 and 2006 found these areas to be endemic for geohelminths (*A. lumbricoides*, hookworms and *T. trichiuria*).

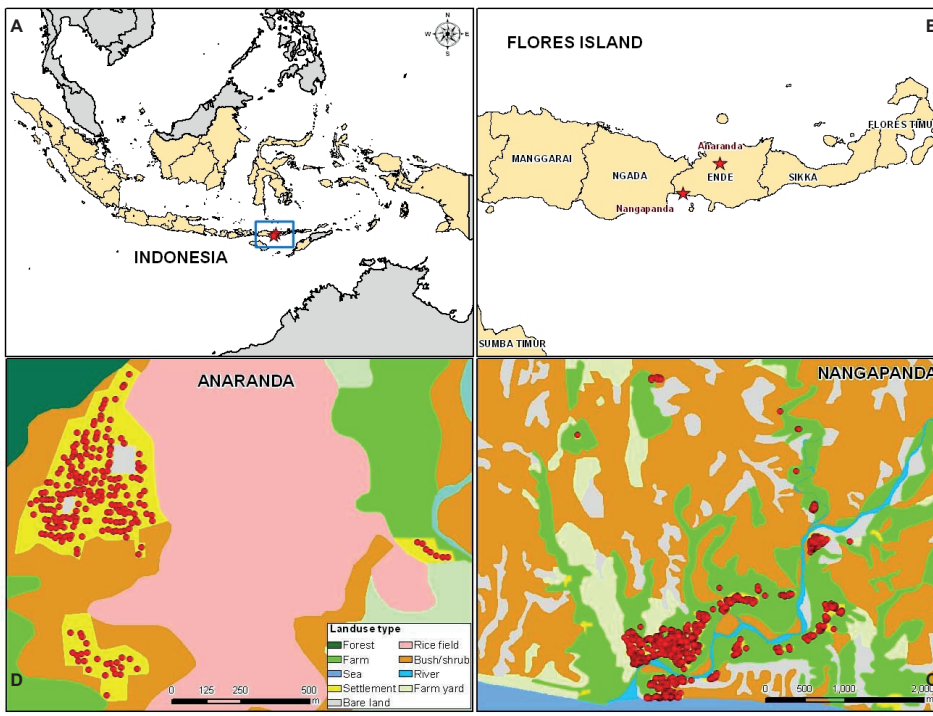


Figure 1. The map of the study area in Flores, Indonesia*. A. Indonesia map. B. Study areas in Flores island which star sign. C. Nangapanda (semi urban area) where each dot represents one household. D. Anaranda (rural area) where each dot represents one household.*

Design

This study of schoolchildren is designed as a double-blind randomized trial with two arms. One arm is treatment with albendazole (single dose of 400 mg), while the other arm is treatment with matching placebo (both tablets from PT Indofarma Pharmaceutical, Bandung, Indonesia). The treatment will be provided every three months for a period of two years (total of 8 treatments). The resident population of the study area were randomized, by computer aided block randomization at household level, using Random Allocation Software[37] to either receive

placebo or treatment. The treatment will be coded with random numbers and the code will be concealed from investigators and patients. Labels with the study subject ID will be printed from a computer database and attached to the appropriate strip of treatment by a separate team located in Jakarta without the involvement of the study investigators. Treatment codes will be unblinded by a monitoring committee after 1 year of treatment for interim analysis of any adverse effects that retention of anthelmintic treatment might have on the growth of children and on the incidence of allergy. If the trial continues, the final unblinding of the codes will take place after two years of treatment. At the end of the study the whole population will be treated.

From the total study population, schoolchildren aged 5-15 years will be included in the study of allergy parameters at pre, 1 and 2 years post treatment. The baseline demographic data as well as detailed questionnaires to delineate risk and protective factors for the development of allergies are planned at pretreatment stage. Skin prick testing (SPT) to allergens, International Study of Asthma and Allergy in Childhood (ISAAC) questionnaires, stool collection for helminth load, blood sampling for serology as well as whole blood culture is planned for time points pre, 1 and 2 years post treatment while exercise-induced bronchialconstriction (EIB) will be measured at pre and two years post treatment. Whereas SPT, ISAAC, parasitological examination as well as serology will be performed for all study subjects, where blood culture will be performed for children randomly selected based on households. The EIB will also be done in a subset of randomly selected children based on helminth as well as SPT status.

Sample size

Data available from a pilot study where 102 schoolchildren were skin prick tested as well as data available from studies on other areas of Indonesia[38,39] were used for sample size calculations. Initial prevalence of SPT reactivity to a panel of allergens was found to be 15%. In order to find a 50% increase or decrease in SPT in the population, and taking into account a loss to follow-up of 20%, 709 individuals were needed in each treatment arm (taking into account a power of 0.90 and an alpha of 0.05). The reported prevalence of SPT to aeroallergens in children rural versus urban areas in low and middle income countries is around 10% versus 20%[31,40]. Moreover, as treatment studies have often shown a doubling of SPT reactivity[4,33], we have based our sample size calculation on a more modest increase as the prevalence of helminth infections is high in Anaranda and Nangapanda areas and therefore it might be difficult to eliminate these infections.

Information, recruitment, consent, specimen collection and storage

The local health authorities in Ende were informed and they gave their agreement and support for this study. Socialization took place over a two-year period, from 2006 to 2008. Staff members from the Puskesmas are being fully involved and 50 community workers are being trained for following study subjects, filling questionnaires and keeping the community well-informed and well-engaged. Through many organized sessions, the village heads are being involved in passing on information about the study, including the benefits and risks involved. The longitudinal nature

is explained and information sheets and consent forms (in Bahasa Indonesia) were distributed. Parents or guardians gave informed consent which was registered by signature or thumb print.

Peripheral blood will be drawn once a year at baseline, 1 and 2 years after treatment for immunological studies. Stool samples will be collected once a year for intestinal helminth examination. Whole blood cultures will be set up using samples from individuals identified in a subset of households randomly selected from the treatment and placebo arms (Figure 2). All blood samples (serum, cell pellet, plasma, and whole blood), blood culture supernatants, as well as stool samples for PCR, will be kept at -20°C and sent to Jakarta on dry ice to be kept at -20°C (plasma, cell plasma, blood culture supernatant) or -80°C (serum). At baseline, we mapped all houses using GPS system. All data that will be collected will be stored in an MS Access database (Microsoft, Redmond, WA, USA).

1. Questionnaires and additional measurements

Additional factors that could influence allergy or atopy will be obtained by questionnaires. The questionnaires include the core allergy symptoms questions of ISAAC and information on history of disease and treatment in the last 12 months, treatment for worm infections, history of immunization, history of breast feeding and food consumption. The questionnaires will be administered to the parent or guardian of each child under supervision of an interviewer.

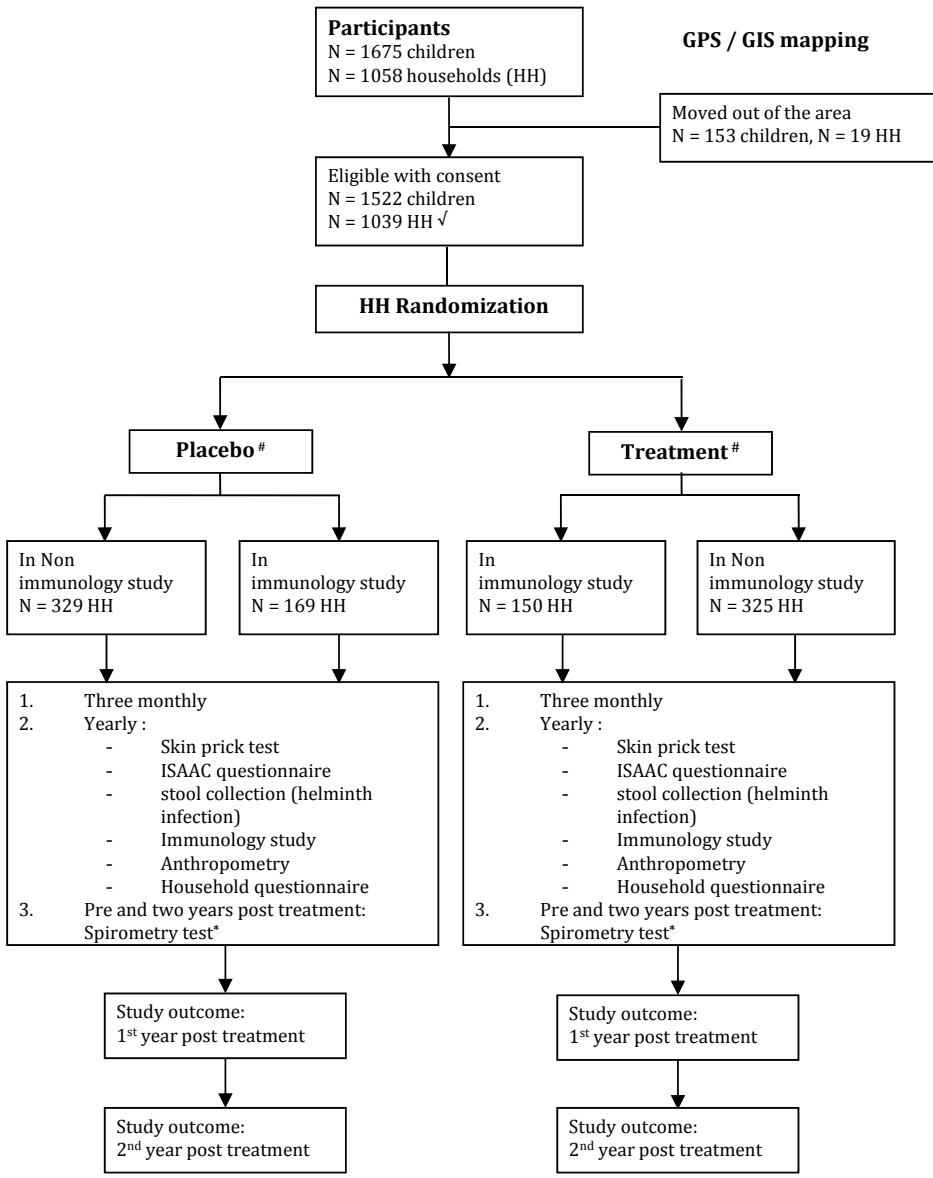
The same interviewer will dispense the ISAAC questionnaire during house to house visits. For core ISAAC questionnaires, we will show a video of asthma and rhinitis as well as pictures for dermatitis. Additional questionnaires will be held on socio-economic status, hygiene, ethnicity and environment factors. These questionnaires include information as material of the house, use of processed water, electricity, floor material, fuel, management of waste and exposure to pets and animals, and parent education level. Standing height and weight without shoes will be measured.

2. Skin prick test

Skin prick test (SPT) reactivity to common aeroallergens[41] will be tested with extracts of *Dermatophagoides pteronyssinus* and *farinae* (HAL Allergy Laboratories, Leiden, The Netherlands) and *Blattella germanica* (Lofarma, Milan, Italy) and to four allergens with extracts of shrimp, soybean, peanut and fish (HAL Allergy). A histamine positive control and a saline negative control will be used to reduce false positives and negatives. SPT will be done on the volar side of the child's lower arm using skin prick lancets (Stallergènes SA, Antony, France). The wheal size will be measured after 15 minutes. Skin prick reactivity is to be considered positive if the longest diameter of the wheal size plus the diameter perpendicular to it divided by two is at least 3 mm. All SPTs in the study will be performed by the same investigator.

3. Spirometric and exercise challenge test

One hundred and twenty children selected on the basis of being helminth positive or negative and SPT positive or negative will be randomized to select equal number of males and females



√ 66 households do not have children 5-15 years old
Three monthly treatment with albendazole or placebo
* in a subset
N number

Figure 2. Profile of the ImmunoSPIN-Allergy subproject (www.immunospin.org) in study areas, Flores, Indonesia.

that fall into the four categories: helminth positive and SPT positive, helminth negative and SPT negative, helminth positive and SPT negative, helminth negative and SPT positive for spirometric test. This test will be performed before exercise, three minutes after exercise and at eight minutes after exercise. Each child will go through a vigorous six minutes free running exercise in the school playground and their heart rate will be recorded before and after exercise as a measurement of the level of exercise stress achieved. Spirometer calibrations will be done daily before use according to ambient atmospheric pressure and temperature. To determine whether EIB has occurred, we will compare the highest forced expiratory volume in one second FEV1 value from at least three acceptable trials before exercise with the lowest FEV1 from at least three acceptable trials after exercise. Spirometric tests will be performed using a portable spirometer (Jaeger, Germany) with the child sitting and with a nose clip. EIB will be defined as positive if FEV1 falls by 15% or more after exercise.

4. Parasitological examination

Stool examination by microscopy

The formol-ether acetate concentration method[42] will be performed on the formalin preserved stool samples followed by microscopic examination for intestinal helminth infections, as well as protozoan infections. For the detection of hookworm larvae, an amount of fresh stool sample will be cultured using filter paper soaked by distilled water inside a sealed plastic bag according to the Harada Mori method and the presence of larvae will be determined by microscopic examination after seven days[43].

Stool examination by real-time PCR

Stool examination by real time PCR will be done in the Netherlands as previously described[36]. Briefly, DNA will be extracted from unpreserved stool samples were stored at -20°C). A multiplex real-time PCR will be used for the specific amplification and detection of *A. duodenale*, *N. americanus*, *A. lumbricoides*, and *S. stercoralis* DNA[44,45]. Amplification reactions will be performed in white PCR plates in a volume of 25 µl with PCR buffer (Hotstar Taq master mix, QIAgen, Germany), 5 mM MgCl2, 2.5 µgram Bovine Serum Albumin (Roche Diagnostics Nederland B.V., Almere, the Netherlands), 5 pmol of each A. duodenale-specific primer, and of each N. americanus-specific primer, 2 pmol of each *A. lumbricoides* specific primer, 2.5 pmol of each *S. stercoralis* specific primer and 3.75 pmol of each PhHV-1-specific primer, 1.25 pmol of each *N. americanus* specific XS-probe, *A. lumbricoides* specific XS-probe, *S. stercoralis* specific double-labelled probe, and PhHV-1-specific double-labelled probe, and 2.5 pmol of the *A. duodenale* specific XS-probe, and 5 µl of the DNA sample.

Amplification consists of 15 min at 95°C followed by 50 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Amplification, detection, and analysis will be performed with the CFX real-time detection system (Bio-Rad laboratories). The PCR output from this system consists of a cycle-threshold (Ct) value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence reflecting the parasite-

specific DNA load in the sample tested. Negative and positive control samples are included in each amplification run.

The amplification is considered to be hampered by faecal inhibitory factors if the expected cycle threshold (Ct) value of 33 in the PhHV-specific PCR is increased by more than 3.3 cycles.

5. Whole blood culture and cytokine measurements in supernatants

Whole blood culture will be undertaken in the field studies and cytokine measurements will be done in Jakarta, Indonesia as described previously[36]. Briefly, the heparinized blood will be diluted 1:4 with RPMI 1640 medium (Invitrogen, Breda, The Netherlands) and cultured in 96 well round bottomed plates. Stimulations will be performed with medium/control, PHA (2 µg/ml, Wellcome Diagnostics, Darford, UK), LPS (1 ng/ml Sigma-Aldrich, Zwijndrecht, The Netherlands), Pam3Cys (100 ng/ml, Cayla-InvivoGen Europe, Toulouse, France), PolyIC (50 µg/ml, Cayla-InvivoGen Europe, Toulouse, France) and *Ascaris* antigen (20 µg/ml as prepared by van Riet E *et al*[46]. Supernatants will be collected on day 1 (unstimulated control, LPS, Pam3Cys) and day 3 (unstimulated control, PHA, Ascaris, PolyIC). TNF-α and IL-10 from day 1 supernatants as well as IL-2, IL-5, IL-10, IFN-γ, and TNF-α for day 3 supernatants will be analysed simultaneously using commercial Luminex cytokine kit (Biosource, Camarillo, CA, USA) and run on a Liquichip 200® Workstation (Qiagen, Venlo, The Netherlands) equipped with Liquichip analyzer software (Qiagen, Venlo, The Netherlands).

6. Antibody measurements

Antibody IgE measurement

Measurement of plasma specific IgE to *A. lumbricoides* antigen and to *D. pteronyssinus* (house dust mite), *B. germanica*, shrimp and peanut will be performed using an ImmunoCAP 250 system (Phadia, Uppsala, Sweden) following the manufacturer's instructions[47]. All measurement will take place in one laboratory in the Netherlands.

Total IgE

Total IgE will be measured in Jakarta, Indonesia as described previously[36,48]. Briefly, maxisorp plates (Thermo Fisher Scientific, Roskilde, Denmark) will be coated overnight with 1/1400 diluted rabbit anti-human IgE (Dako, Glostrup, Denmark). Plates are blocked with phosphate buffered saline (PBS) containing 5% bovine serum albumin (BSA, Albumin Fraction V, Boehringer, Mannheim, Germany). Sera are diluted 1/200 in PBS containing 5% fetal calf serum (FCS, Greiner Bio-One, Alphen a/d Rijn, Netherlands). A positive standard serum containing human IgE (NIBSC, Potters Bar, UK) is incubated on each plate. Plates will be incubated for 1 hour at room temperature. After a washing step, IgE biotinylated goat anti-human IgE antibody (1/1000 (Vector Laboratories, Burlingame, CA, USA)) and Streptavidin Alkaline Phosphatase conjugate (1/3000 (Boehringer, Mannheim, Germany)) will be incubated for 1 hour at room temperature. The results will be expressed in International Units (IU/ml).

Outcomes and case definitions

The study aims to determine whether and how helminth infections may affect allergic disorders. The study will therefore determine the effect that anthelmintic treatment albendazole has versus placebo on SPT reactivity, symptoms of allergic diseases and immune responses. The primary outcome of the study is SPT; the secondary outcome is symptoms (asthma and atopic dermatitis); while the tertiary outcome is immune responses (both IgE levels to allergens and cellular immune responses that represent both innate and adaptive immune reactivities).

Helminth infections will be determined by the presence of parasites detected by microscopic examination of stool as well as by molecular (PCR-based) methods. Atopy will be defined as either positive in SPT (≥3 mm wheal size) to any of aeroallergens tested or the presence of allergen-specific IgE ≥ 0.35 kU_A/L. Symptoms of asthma and atopic dermatitis will be assessed by modified ISAAC questionnaires[41] that have been translated into Bahasa Indonesia and translated back to English and adapted for use in the study area. It has been tested in some areas in Sulawesi as well as in study areas during pilot studies. A positive answer to the questions (i) has your child ever had asthma? (ii) has this asthma been diagnosed by a doctor? and (ii) has your child ever or in the past 12 months had wheezing or whistling in the chest? will be interpreted as asthma, while a positive answer to the question (iv) has your child ever had doctor/paramedic diagnosed allergic eczema and (v) has your child ever had one or more skin problem accompanying an itchy rash? will be taken as atopic dermatitis.

In order to test the effect of helminths on bronchial hyper-responsiveness (BHR) we will test lung function following American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines[49] for lung function and BHR testing[41,50,51]. FEV1 will be measured before and after 6 minutes of exercise[52] and a fall of 15% in FEV1 is considered to indicate EIB. In order to take into account confounding factors, data on family structure like number of siblings and birth order will be recorded, as well as details of birth and breastfeeding, hygiene, socio-economic status, annual health status, and food consumption.

Overview of plan of analyses

The baseline data will be analyzed to determine whether helminth infections are associated with allergen specific IgE, atopy and symptoms of asthma or eczema. Both presence of infection and intensity of infection will be used for logistic and linear regression analyses. Analysis will be adjusted for confounding factors such as socio-economic status, body mass index, age, sex and environmental exposure. Additional confounders that are identified during the study will also be used. In order to examine a general effect of parasite burden and rural and semi-urban differences in atopy and allergy will be compared. The effect of anthelmintic treatment on atopy and allergy will be assessed 1 and 2 years after treatment by analyzing prevalence as well as incidence. The analyses will be based on intention to treat approach. The groups in treatment and placebo arms will be compared as well as groups in whom helminth infection was reduced or remained unaltered irrespective of treatment assignment and we will also look at chronic versus acute infections, based on continuous

presence of infection or newly gained infections over the follow up period. Individuals that are lost to follow up and individuals that are analyzed will be compared on the basis of their baseline characteristics, age, gender, village, socio-economic status and parasitic infections. A similar comparison will also be undertaken to compare the characteristics of individuals in the treatment and placebo groups at inclusion into the study. chi-square, *t*-tests and Mann-Whitney tests will be used to test for differences. For data-analyses we will use SPSS (SPSS Inc., Chicago, IL, USA) and ArcGis (ESRI; Redlands, CA, USA).

To summarize, the plan is to measure the prevalence of allergy in school-aged children in semi urban and rural areas, and to establish their association with helminth infections as well as various risk and protective factors. By studying cellular immunological parameters, it is also the aim to understand the immunological mechanisms that are behind risk and protective factors in allergy.

The analysis will be divided into six principals study questions (Figure 3):

1. What is the association between helminth infections and atopy and allergy symptoms?
2. What is the association of helminth infections and immune responses?
3. What is the association between immune responses and atopy and allergy symptoms?
4. Does the immune response change after anthelmintic treatment?
5. Does the prevalence of atopy and allergy symptoms change after anthelmintic treatment?
6. What is the role of hygiene, geographic location and socio-economic status in helminth infection and allergy?

Ethical consideration and trial registration

The study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia, Jakarta (ethical clearance ref: 194/PT02.FK/Etik/2006) and has been filed by ethics committee of the Leiden University Medical Center, The Netherlands. The trial was

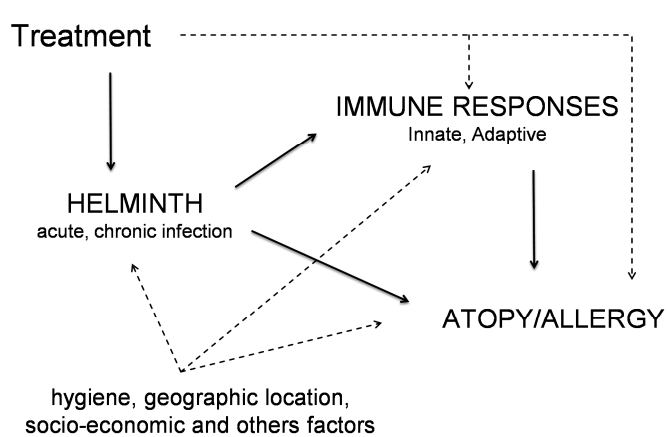


Figure 3. Conceptual of principal question study.

registered as clinical trial ref: ISRCTN83830814. Parental consent was obtained for children who participated in the study. The study is reported in accordance with the CONSORT guidelines for cluster-randomized studies.

DISCUSSION

The prevalences of atopic disorders and asthma have been reported to be lower in low income than in high income countries, however these are becoming an increasingly important public health problem, particularly in urban centres of the developing world[35,53]. With the view to the increasing urbanization it is important to have data on factors and mechanisms underlying the development of allergic diseases in low to middle income countries. Indonesia is a prototype of a country in transition towards a well-developed economy with dynamic changes in lifestyle. The ImmunoSPIN project was designed to assess how helminth infections can influence the immune response and clinical outcome of allergic disease and this study will compare atopy and allergic symptoms in children living in a semi urban and rural setting in Indonesia. The relations between allergic outcomes and the numerous measured exposures will be addressed.

In our study design we will use a longitudinal approach to assess the effect of anthelmintic treatment on prevalence, risk and protective factors in children living in semi urban and rural environments. The laboratory component will explore the relative importance of immunological mechanisms that are leading to increase in prevalence of allergic disorders. Statistical analysis will involve the use of strategies that link data from different levels (e.g. socio-economic, environmental, clinical and immunological factors) and will use advanced statistical techniques to deal with this complex mechanism.

The study has so far provided data that are shown in a flow chart given in Figure 2. A total of 1675 children were registered in 1058 households in study area, characteristics of the study population are given in table 1 with prevalence of helminth infection in both areas.

Distribution of sex and age groups are shown in (Figure 4) in the semi urban area which has three junior high schools and four elementary schools, as well as in the rural area which has only one elementary school. This results in a larger number of older children in semi urban area than in rural area. Some of the children from rural area leave the area to go to junior high school in surrounding larger villages but not necessarily to our semi urban study area (as the distance between our study areas is considerable). These children will be skin prick tested, and characterized via questionnaires.

In addition, blood withdrawal will allow the determination of total and specific IgE in addition to cellular immunological measurements and in a sub sample the spirometric test will be performed. After completion of the study the whole population will be given adequate treatment for helminth infections. This study is also unique in that it will provide data on anthelmintic treatment efficacy and effectiveness in a defined large population in a developing country.

If helminth infections are proved to be associated with reduced allergic disorders, and the mechanisms behind such protective effect is elucidated, measures can be taken to ensure that the vicious circle of westernization and increased allergy is prevented; for example vaccination

Table 1. Characteristics of study population

	Semi Urban	Rural
Participants	1161	514
Age (mean, SD)	6.77 ± 3.31	5.85 ± 3.12
Sex, male/female (male%)	583/578 (50.2)	270/244 (52.5)
BMI (mean, SD)	15.4 ± 2.39	14.4 ± 1.83
Parasites (%)		
Any helminths	53.2	40.8
Hookworms	11.8	18.5
Ascaris lumbricoides	38.7	21.8
Trichuris trichiura	25.3	11.8

Body Mass Index (BMI) = Weight (kg)/height(m) squared. Helminth infection was measured by microscopic examination

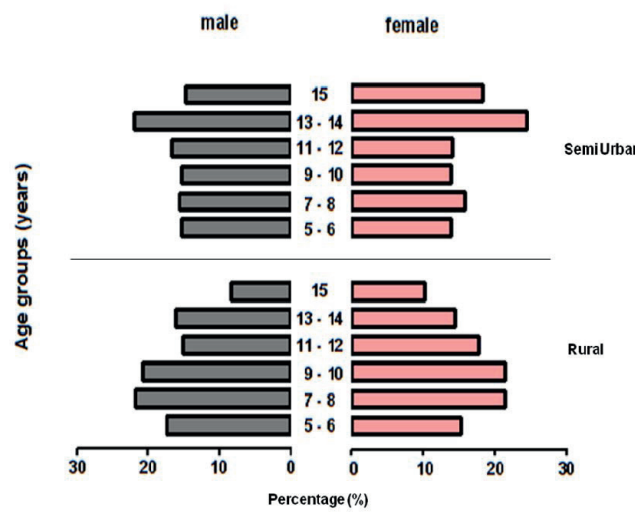


Figure 4. Distribution of sex and age in school children participating in the study. *Distribution of children participants in the semi urban (Nangapanda) and the rural (Anaranda) areas.*

with microbial products and allergens. If helminths are proved not to be associated but new factor(s) are identified along with the immunological mechanism(s) through which the development of allergies is affected, then appropriate preventive measures can also be planed.

In summary the ImmunoSPIN helminth-allergy study is the first and currently the only longitudinal study of helminth and allergy in Indonesia. The study has received enthusiastic support from the authorities in Ende and at the regional level. At the same time, the study facilitates the transfer of state of the art technologies in immunology, molecular biology, epidemiology and statistics to Indonesia.

REFERENCES

1 Bethony J, Brooker S, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521-32.

2 Cooper PJ. Mucosal immunology of geohelminth infections in humans. Mucosal Immunol 2009;2:288-99.

3 Grencis RK, Hultner L, et al. Host protective immunity to Trichinella spiralis in mice: activation of Th cell subsets and lymphokine secretion in mice expressing different response phenotypes. Immunology 1991;74:329-32.

4 Flohr C, Tuyen LN, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2010;40:131-42.

5 Everts B, Perona-Wright G, et al. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. J Exp Med 2009;206:1673-80.

6 Steinfelder S, Andersen JF, et al. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). J Exp Med 2009;206:1681-90.

7 Royer PJ, Emara M, et al. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. J Immunol 2010;185:1522-31.

8 Cooper PJ. The potential impact of early exposures to geohelminth infections on the development of atopy. Clin Rev Allergy Immunol 2004;26: 5-14.

9 Cooper PJ. Intestinal worms and human allergy. Parasite Immunol 2004;26:455-67.

10 Flohr C, Quinnell RJ, et al. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009;39:20-32.

11 Cooper PJ. Can intestinal helminth infections (geohelminths) affect the development and expression of asthma and allergic disease? Clin Exp Immunol 2002;128:398-404.

12 Erb KJ. Helminths, allergic disorders and IgE-mediated immune responses: where do we stand? Eur J Immunol 2007;37:1170-3.

13 Yazdanbakhsh M, van den Biggelaar A, et al. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001;22:372-7.

14 Yazdanbakhsh M, Wahyuni S. The role of helminth infections in protection from atopic disorders. Curr Opin Allergy Clin Immunol 2005;5:386-91.

15 Lynch NR, Hagel I, et al. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 1993;92:404-11.

16 van den Biggelaar AH, van Ree R, et al. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. Lancet 2000;356:1723-7.

17 Dagoye D, Bekele Z, et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am J Respir Crit Care Med 2003;167:1369-73.

18 Cooper PJ, Chico ME, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 2003;111:995-1000.

19 Smits HH, Hammad H, et al. Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol 2007;120:932-40.

20 Wilson MS, Taylor MD, et al. Helminth-induced CD19+CD23hi B cells modulate experimental allergic and autoimmune inflammation. Eur J Immunol 2010;40:1682-96.

21 Amu S, Saunders SP, et al. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J Allergy Clin Immunol 2010;125:1114-24.

22 Yazdanbakhsh M, Kremsner PG, et al. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.

23 Nookala S, Srinivasan S, et al. Impairment of tetanus-specific cellular and humoral responses following tetanus vaccination in human lymphatic filariasis. Infect Immun 2004;72:2598-2604.

24 Satoguina JS, Adjobimey T, et al. Tr1 and naturally occurring regulatory T cells induce IgG4 in B cells through GITR/GITR-L interaction, IL-10 and TGF-beta. Eur J Immunol 2008;38:3101-13.

25 Wilson MS, Taylor MD, et al. Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 2005;202:1199-212.

26 Selassie FG, Stevens RH, et al. Total and specific IgE (house dust mite and intestinal helminths) in

asthmatics and controls from Gondar, Ethiopia. Clin Exp Allergy 2000;30:356-8.

27 Davey G, Berhane Y, et al. Use of acetaminophen and the risk of self-reported allergic symptoms and skin sensitization in Butajira, Ethiopia. J Allergy Clin Immunol 2005;116:863-8.

28 Palmer LJ, Celedon JC, Weiss ST, Wang B, Fang Z, Xu X: Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002;165:1489-93.

29 Haileamlak A, Lewis SA, et al. Validation of the International Study of Asthma and Allergies in Children (ISAAC) and U.K. criteria for atopic eczema in Ethiopian children. Br J Dermatol 2005;152:735-41.

30 Ait-Khaled N, Pearce N, et al. Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. Allergy 2009;64:123-48.

31 Addo-Yobo EO, Woodcock A, et al. Exercise-induced bronchospasm and atopy in Ghana: two surveys ten years apart. PLoS Med 2007;4:e70.

32 Steinman HA, Donson H, et al. Bronchial hyper-responsiveness and atopy in urban, peri-urban and rural South African children. Pediatr Allergy Immunol 2003;14:383-93.

33 van den Biggelaar AH, Rodrigues LC, et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.

34 Cooper PJ, Chico ME, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006;367:1598-603.

35 Weinmayr G, Genuneit J, et al. International variations in associations of allergic markers and diseases in children: ISAAC Phase Two. Allergy 2010;65:766-75.

36 Wiria AE, Prasetyani MA, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2010;10:77.

37 Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol 2004;4:26.

38 Supali T, Djuardi Y, et al. Relationship between Different Species of Helminths and Atopy: A Study in a Population Living in Helminth-Endemic Area in Sulawesi, Indonesia. Int Arch Allergy Immunol 2010;153:388-94.

39 Wahyuni S, Sartono E, et al. Clustering of allergic outcomes within families and households in areas endemic for helminth infections. Int Arch Allergy Immunol 2005;136:356-64.

40 Hartgers FC, Obeng BB, et al. Lower expression of TLR2 and SOCS-3 is associated with Schistosoma haematobium infection and with lower risk for allergic reactivity in children living in a rural area in Ghana. PLoS Negl Trop Dis 2008;2:e227.

41 Weiland SK, Bjorksten B, et al. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. Eur Respir J 2004;24:406-12.

42 Allen AV, Ridley DS. Further observations on the formol-ether concentration technique for faecal parasites. J Clin Pathol 1970;23:545-6.

43 Harada Y, Mori O. A new method for culturing hookworm. Yonago Acta Medical 1955, 177-9.

44 Verweij JJ, Canales M, et al. Molecular diagnosis of Strongyloides stercoralis in faecal samples using real-time PCR. Trans R Soc Trop Med Hyg 2009;103:342-6.

45 Verweij JJ, Brienen EA, et al. Simultaneous detection and quantification of Ancylostoma duodenale, Necator americanus, and Oesophagostomum bifurcum in fecal samples using multiplex real-time PCR. Am J Trop Med Hyg 2007;77:685-90.

46 van Riet E, Wuhrer M, et al. Antibody responses to Ascaris-derived proteins and glycolipids: the role of phosphorylcholine. Parasite Immunol 2006;28:363-71.

47 Johansson SG. ImmunoCAP Specific IgE test: an objective tool for research and routine allergy diagnosis. Expert Rev Mol Diagn 2004;4:273-9.

48 Terhell AJ, Stolk WA, et al. Regulation of anti-filarial IgE by infection pressure. Parasitology 2002;124:509-19.

49 Miller MR, Hankinson J, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.

50 Haby MM, Anderson SD, et al. An exercise challenge protocol for epidemiological studies of asthma in children: comparison with histamine challenge. Eur Respir J 1994;7:43-9.

51 Ponsonby AL, Couper D, et al. Exercise-induced bronchial hyperresponsiveness and parental

ISAAC questionnaire responses. Eur Respir J 1996;9:1356-62.

52 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.

53 Weinmayr G, Weiland SK, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2007;176:565-74.

4

RISK FACTORS ASSOCIATED WITH THE DEVELOPMENT OF ATOPIC SENSITIZATION IN INDONESIA

Firdaus Hamid[1,2], Aprilianto E Wiria[2,3], Linda J Wammes[2], Maria MM Kaisar[2,3], Yenny Djuardi[3], Serge A Versteeg[4], Sitti Wahyuni[5], Ronald van Ree[4,6], Erliyani Sartono[2], Taniawati Supali[3], Maria Yazdanbakhsh[2]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
4. Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
5. Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
6. Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

PLoS ONE 2013;8:e67064

ABSTRACT

Background The prevalence of allergic diseases has increased not only in high income but also in low-to-middle income countries. However, risk factors for their development are still not well established, particularly in the latter.

Objective To assess prevalence and identify risk factors for sensitization to two major inhalant allergens among children from semi-urban and rural areas in Indonesia.

Method A cross-sectional survey was performed among 1,674 school children aged 5–15 years old. Information on potential risk factors and reported allergic symptoms were obtained by questionnaire. Helminth infections were assessed. Skin prick tests (SPT) were performed, total IgE as well as allergen-specific IgE for house dust mite (HDM) and cockroach were measured.

Result The prevalence of allergic skin sensitization to both aeroallergens was significantly higher in the semi-urban than in the rural area. However, serum IgE against HDM and cockroach as well as total IgE were significantly lower in semi-urban than in rural children. In the semi-urban area, there was a significant positive association between SPT to HDM and higher paternal education but a negative one with hookworm infection. The risk factors linked to cockroach sensitization were different: being of a farmer offspring and lacking access to piped water were associated with an increased risk for a positive SPT to cockroach. No significant associations between measured risk factors and having a positive SPT were found in the rural area.

Conclusion Sensitization to HDM and cockroach is common in Indonesia, more often translating into a positive SPT in the semi-urban than in the rural setting. Whereas high paternal education and low hookworm infection were associated with increased risk of a positive SPT to HDM, we were surprised to find that parameters of lower SES were identified as risk factor for a positive cockroach SPT.

INTRODUCTION

The prevalence of allergic diseases has increased not only in high income but also in low-to-middle income countries (LMIC)[1]. However, in the LMIC, whereas high prevalence of allergies is seen in urban centers, allergic disorders are usually rare in rural areas[2,3].

It is well-established that the development of allergic disorders is the result of a complex interplay of genetic background and environmental factors[4]. Various exogenous factors including parental smoking, allergen exposure, and outdoor and indoor air pollution have been shown to be associated with an increase in the prevalence of allergic disease, while other factors such as exposure to infections in early childhood, a 'traditional' diet or lifestyle, and contact with animals were shown to reduce the risk of developing allergic disorders[5].

With respect to infections, parasitic helminths have often been shown to be negatively associated with allergic outcomes[6]. Previous studies have shown that having *Schistosoma*[7] or filarial infection[8] decrease the risk of skin prick test (SPT) positivity. Similarly, studies in Ecuadorian[9] and Vietnamese[10] children showed that having soil-transmitted helminth infections decrease the risk of atopy. In these studies, it was also clear that other factors such as overcrowding in household, poor sanitation as well as low socio-economic status can be associated with less atopy[9,10]. In general, helminth infections are associated with poor sanitation, low socio-economic status and rural setting in low income countries[11], therefore dissecting the relative contribution of the different factors will be important in understanding the rise in allergies worldwide[12].

Indonesia is one of the LMIC which is in epidemiological transition with increasing level of urbanization accompanied by changing disease patterns. Risk factors for the development of allergic disorders are still not well established in Indonesia. Therefore, we set up a study in a semi-urban and a rural area on Flores Island, in Indonesia, to estimate the prevalence of allergic sensitization to two major respiratory allergen sources (house dust mite and cockroach) and to identify associated risk factors.

METHODS

Study area and design

This cross-sectional study was performed between January and June 2008 in Ende District of Flores Island, Indonesia, as part of a longitudinal study investigating the effect of anthelminthic treatment on malarial parasitemia and allergy, described in detail elsewhere[13,14]. In a preliminary study, we surveyed several areas in Ende district and found that both semi-urban, Nangapanda, and rural, Anaranda, were endemic for soil-transmitted helminth. A total of 1674 (1161 and 513 from semi-urban and rural, respectively) school children aged 5 – 15 years were included in this study. Allergic disorders were assessed by a questionnaire slightly modified from the validated questionnaire used by the International Study of Asthma and Allergy in Childhood (ISAAC) to accommodate local study requirements. Questionnaires were administered to obtain demographic data, socio-economic status and other potential risk or protective factors for the development of allergies. In addition, skin reactivity (by SPT)

and specific IgE (by ImmunoCAP) to house dust mite (HDM) and cockroach as well as total IgE (by ELISA) were measured. Stool was collected for determination of helminth infection. Written informed consent was obtained from parent or guardian of each child. The study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia, Jakarta (ethical clearance ref: 194/PT02.FK/Etik/2006) and has been filed by ethics committee of the Leiden University Medical Center, The Netherlands.

Questionnaires

Reported clinical symptoms of allergy were obtained by questionnaire. The interview was conducted with the parent or guardian of the children. History of asthma and atopic dermatitis (eczema) were assessed using a modified ISAAC questionnaire, which had been translated into Bahasa Indonesia. The prevalence of asthma symptoms were obtained from the following questions: (i) Has your child ever had wheeze? (ii) Has this asthma been diagnosed by a doctor? and (iii) Has your child ever or in the past 12 months had wheezing or whistling in the chest?; while eczema was obtained from the questions: (i) has your child ever had doctor diagnosed allergic eczema and (ii) has your child ever had one or more skin problems accompanied by an itchy rash?.

An additional questionnaire was administered to obtain data on demographic, parental education, parental occupation, water supply, living condition of families and other characteristics of the households. Educational levels were categorized as: low for illiterate or elementary school and high for secondary school or above. The majority reported parental occupation as 'farmers'; therefore occupation was categorized into farmers and non-farmers.

Skin prick testing

SPT reactivity to common aeroallergens was tested with extracts of HDM (*Dermatophagoides pteronyssinus* and *D. farinae;* HAL Allergy Laboratories, Leiden, The Netherlands) and cockroach (*Blattella germanica*; Lofarma, Milan, Italy). Histamine and allergen diluents were used as positive and negative controls, respectively. SPT was done on the volar side of the lower arm. The wheal size was measured after 15 minutes. Skin prick reactivity was considered to be positive if the longest diameter of the wheal size plus the diameter perpendicular to it divided by two was at least 3 mm. Individuals with at least one positive reaction to either *D. pteronyssinus* or *D. farinae* were classified to be positive for SPT to HDM. The same investigator performed all the SPTs. Body weight and height were also measured.

Specific and Total IgE

The levels of specific IgE (sIgE) to *D. pteronyssinus* and *B. germanica* were measured in plasma using an ImmunoCAP 250 system, (Thermo Fisher Scientfic, Uppsala, Sweden) following the manufacturer's instructions. All measurements were conducted in one laboratory in The Netherlands.

The levels of total IgE were measured by ELISA in Jakarta as described previously[13,14]. The results were expressed in International Units (IU/ml).

Parasitological examination

The formol-ether acetate concentration method was performed on formalin-preserved stool samples followed by microscopic examination for detection of *Trichuris trichiura*. Aliquots of unpreserved stool samples were kept frozen at −20°C. DNA detection of the parasite in the stool samples was performed in the Netherlands. A multiplex real-time PCR was used for the specific DNA amplification and detection of hookworm (*Ancylostoma duodenale* and *Necator americanus), Ascaris lumbricoides and Strongyloides stercoralis* as has been described in detail previously[13]. PCR output was expressed as the cycle threshold (Ct) reflecting the load of parasite specific DNA in the sample tested. Parasite specific DNA loads of *N. americanus* and *A. lumbricoides* were categorized in low load (Ct≥30) and high load (Ct<30).

Statistical analysis

We investigated risk factors for allergic disorders separately for each area. Descriptive data were expressed as means (± standard deviations), geometric means [95% confidence intervals (CI)] and frequencies (percentage of collected data). Prevalence rates were calculated and compared for different areas using Pearson chi-Square tests. Age-standardized z-scores of body mass index (z-BMI) were calculated according to WHO references[15]. Log transformation of sIgE and total IgE was used to obtain normally distributed data. Student *t*-tests were used for comparison the differences between the outcomes studied in the two populations. The associations between the risk factors and development of positive SPT and reported clinical symptoms of allergy were tested by logistic regression. Linear regression was used for analysis of continuous outcomes which provided estimated regression coefficients (β) and their corresponding 95% CI. In multivariate analysis, we included age and sex as *a priori* confounders, as well as other variables that were previously significant in univariate analyses. We retained in the model those for which there was significant heterogeneity across categories, taking $P < 0.05$ as statistically significant. In the case of collinear variables of risk factors, we fitted each in the absence of the other and retained in the model the variable with the strongest effect. The collected data were analyzed using PASW Statistics for Windows, version 18.0 (SPSS Inc, Chicago, IL, USA) software. The risk factors of sensitization to HDM and cockroach were different; we have presented the results for each allergen separately.

RESULTS

Demographics and allergic disorders in the study population

Data were collected from 1161 and 513 school children in semi-urban and rural areas, respectively. Children were slightly older (mean age 10.6 years) in the semi-urban compared

to rural area (9.7 years) ($P < 0.001$). The percentage of children reported to have a history of wheeze was 4.1% (38 out of 927) and 1.1% (5 out of 453) in the semi-urban and rural areas, respectively. Only 16 out of 927 (2.3%) children in the semi-urban area and none in rural area reported doctor-diagnosed asthma. In the semi-urban area, eczema was reported in 3.1% of the children (Table 1), while no case was reported in the rural area.

In the semi-urban area the prevalence of a positive SPT was higher than in the rural area for both HDM (13.5% versus 8.5%; $P = 0.007$; respectively) and cockroach (23.8% vs 18.3%; $P = 0.021$; respectively). In contrast, the levels of sIgE to HDM and cockroach as well as total IgE were lower in the semi-urban than in the rural area (Table 1).

Rural population were very homogeneous with regards to socio-economic conditions: parental education was low (89%), farming was the major occupation (95%), 92% (473/513) of houses were made up of bamboo, almost all children had helminth infection and a very high proportion of children (78%) had high load of hookworm infection. In contrast, the semi-urban area demonstrated more heterogeneity with 41% (314/760) of parents having high education, 62% (579/938) being farmers, 43% (143/332) of children having a high load of hookworm infection whereas 87% children had helminth infections.

Table 1. Characteristics of population and allergic disorders in semi-urban and rural areas

	Semi Urban		Rural		
	N	**Result**	**N**	**Result**	***P*-value**
Age years (mean, SD)	1161	10.6 ± 3.3	513	9.7 ± 3.1	**<0.001**$^{\$}$
Sex (N, %)					
Male	583	50.2	272	53.0	0.29$^{\infty}$
Female	578	49.8	241	47.0	
Clinical symptoms of allergy (N, n%)					
Asthma					
History of wheeze	927	38 (4.1)	453	5 (1.1)	**0.0026**$^{\infty}$
Diagnosed-asthma by doctor	927	16 (2.3)	453	0	
Wheeze in the last 12 months	927	15 (1.1)	453	3 (0.7)	0.14$^{\infty}$
Eczema	927	29 (3.1)	453	0	
Skin prick test reactivity (N, n%)					
HDM	975	132 (13.5)	447	38 (8.5)	**0.0066**∞
Cockroach	975	232 (23.8)	447	82 (18.3)	**0.021**∞
Specific IgE and Total IgE (geometric mean, 95 CI)					
HDM$^{\#}$ (kU_A/L)	592	0.66 (0.58-0.76)	292	1.13 (0.95-1.34)	**< 0.001$****
Blattella germanica (kU_A/L)	592	1.33 (1.15-1.54)	292	1.66 (1.38-2.00)	0.074$^{\$}$
Total IgE (IU/ml)	540	1896.9 (1696.8-2120.6)	340	4141.1 (3654.7-4692.3)	**< 0.001$****

SD: standard deviation. The number of positives (n) of the total population examined (N). $^{\#}$IgE to *Dermatophagoides pteronyssinus* (HDM). The statistically significant results are given in bold. $^{\$}$*P*-value derived from Student *t*-test. $^{\infty}$*P*-value derived from Chi-Square test.

Risk factor associated with reported clinical symptoms of allergy

No association was found between measured risk factors and history of wheeze, wheeze in the last 12 months or eczema (data not shown).

Risk factors associated with skin prick test reactivity

In the semi-urban area, the skin reactivity to HDM was positively associated with high paternal education (OR, 1.68; 95% CI, 1.12–2.51) while a high load of *N. americanus* infection was associated with a reduced risk of HDM skin reactivity (OR, 0.47; 95% CI, 0.23–0.95) (Table 2). The odds for positive skin reactivity to cockroach was significantly lower in children who had access to piped water (OR 0.73; 95% CI, 0.54–0.98) but was higher in children whose parents were farmers (OR 1.52; 95% CI, 1.07–2.17) (Table 2).

None of the measured socio-economic factors, z-BMI or helminth infection status was associated with skin reactivity in the rural area (Table S1).

Risk factors associated with total and allergen-specific IgE

The risk factors for sIgE were very different from what was seen for SPT. There was no significant association between risk factors measured and the level of sIgE to HDM in the semi-urban area (Table 3). In the rural area, high load of N. americanus infection was associated with higher levels of sIgE to HDM (β, 0.25; $P = 0.040$) (Table S2).

The examination of cockroach sIgE revealed that in the semi-urban area, high parental education, high socio-economic status (SES) as represented by housing material, cooking fuel and type of floor as well as high z-BMI showed a significantly negative association with sIgE to cockroach. The high load of *A. lumbricoides* and having farmer parents were associated with high levels of sIgE to cockroach (Table 3). The levels of cockroach sIgE tended to be higher in children with high load of *N. americanus* infection in both semi-urban and rural areas (Table 3 and Table S2).

With respect to the levels of total IgE, in the semi-urban but not in the rural area elevated levels of total IgE were associated with having farmer families as well as high load of *N. americanus* or *A. lumbricoides* infections (Table 3). High maternal education was associated with lower levels of total IgE in both semi-urban (β, -0.20; $P < 0.001$) and rural (β, -0.15; $P = 0.046$) areas. The levels of total IgE were significantly lower in children with higher z-BMI (β, -0.06; $P = 0.010$) in the semi-urban area (Table 3). In rural area, using gas/kerosene as cooking fuel was associated with lower levels of total IgE (β, -0.32; $P = 0.030$) (Table S2).

Multivariate analysis

In the semi-urban area, multivariate analysis revealed that *N. americanus* infection tended to reduce the risk of skin reactivity to HDM (adjusted OR, 0.46; 95% CI, 0.21–1.00; $P = 0.051$) (Table 4). In this model, paternal education did not remain as a significant predictor of skin reactivity to HDM. Cockroach skin reactivity was increased in children with farmer parents

Table 2. Association between skin reactivity to HDM or cockroach and potential risk factors for atopy in the semi-urban area[a]

		HDM		Cockroach	
	N	**n (%)**	**OR [95% CI]**	**n (%)**	**OR [95% CI]**
z-BMI (mean, SD)	975	-1.27 ± 1.05[b]	0.95 [0.80-1.13]	-1.27 ± 1.05[b]	0.93 [0.81-1.07]
Paternal education					
Low	442	46 (10.4)	reference	103 (23.3)	reference
High	411	67 (16.3)	1.68 [1.12-2.51]*	89 (21.7)	0.91 [0.66-1.26]
Maternal education					
Low	581	75 (12.9)	reference	151 (26.0)	reference
High	347	54 (15.6)	1.24 [0.85-1.82]	73 (21.0)	0.76 [0.55-1.04]
Parental job					
Non farmer	300	43 (14.3)	reference	55 (18.3)	reference
Farmer	502	65 (12.9)	0.89 [0.59-1.35]	128 (25.5)	1.52 [1.07-2.17]*
House material					
Bamboo / Wood	703	89 (12.7)	reference	175 (24.9)	reference
Stone	272	43 (15.8)	1.30 [0.87-1.92]	57 (21.0)	0.80 [0.57-1.12]
Water source					
Non piped	445	68 (15.3)	reference	120 (27.0)	reference
Piped	530	64 (12.1)	0.76 [0.53-1.10]	112 (21.1)	0.73 [0.54-0.98]*
Toilet					
No	88	10 (11.4)	reference	27 (30.7)	reference
Yes	887	122 (13.8)	1.24 [0.63-2.47]	205 (23.1)	0.68 [0.42-1.10]
Floor material					
Mud	163	17 (10.4)	reference	47 (28.8)	reference
Cement / ceramic	812	115 (14.2)	1.42 [0.83-2.43]	185 (22.8)	0.73 [0.50-1.06]
Fuel					
Wood	827	116 (14.0)	reference	204 (24.7)	reference
Gas / kerosene	148	16 (10.8)	0.74 [0.43-1.29]	28 (18.9)	0.71 [0.46-1.11]
Using sandals					
No	608	79 (13.0)	reference	146 (24.0)	reference
Yes	313	45 (14.4)	1.12 [0.76-1.67]	75 (24.0)	1.00 [0.72-1.37]
N. americanus[1]					
Low load	180	31 (17.2)	reference	49 (27.2)	reference
High load	135	12 (8.9)	0.47 [0.23-0.95]*	25 (18.5)	0.61 [0.35-1.05]
A. lumbricoides[1]					
Low load	200	28 (14.0)	reference	49 (24.5)	reference
High load	115	15 (13.0)	0.92 [0.47-1.81]	25 (21.7)	0.86 [0.49-1.48]
T. trichiura[2]					
Negative	288	32 (11.1)	reference	67 (23.3)	reference
Positive	190	32 (16.8)	1.62 [0.96-2.75]	51 (26.8)	1.21 [0.79-1.84]

[a]association based on univariate logistic model. [b]Mean and standard deviation. [1]diagnosed by PCR. [2]diagnosed by microscopy. The number of positives (n) of the total population examined (N). OR: Odds ratio, CI: Confidence intervals. *$P < 0.05$

(adjusted OR, 1.53; 95% CI, 1.07–2.20; $P = 0.019$), but the negative association with piped water was no longer significant (Table 4).

Children from farmer parents had higher levels of sIgE to cockroach (adjusted β, 0.24; $P = 0.021$) but high z-BMI and *A. lumbricoides* load were no longer associated with sIgE (Table 4). Independently, the levels of total IgE were significantly increased with high load of *N. americanus* as well as high load of *A. lumbricoides* infection (adjusted β, 0.33; $P < 0.001$: adjusted β, 0.22; $P = 0.023$, respectively) (Table 4).

Multivariate analysis in the rural area showed that high load of *N. americanus* infection was still associated with high levels of HDM sIgE (adjusted β, 0.27; $P = 0.031$) after adjusting with *a priori* confounders (Table S3). The levels of total IgE in analysis adjusted for age and sex were significantly lower if kerosene/gas was used as cooking fuel compared to wood (Table S3).

DISCUSSION

The present study showed higher prevalence of positive SPT in semi-urban compared to rural school children on Flores Island in Indonesia. This was accompanied by more reported allergy symptoms. Our finding on rural versus urban differences are in line with other studies showing higher allergies in urban than in rural areas[3,16]. Populations in rural areas often have helminth infections, traditional life style and lower socio-economic status. Here we assessed and determined the relative influence of these different factors. Our major finding in the semi-urban area was that although high paternal education was associated with increased risk of skin reactivity to HDM in univariate analysis, in multivariate it was found that only hookworm infection was an independent protective factor for HDM reactivity. This is consistent with a study in Vietnam[10] which found that helminth infections were independent protective factor for HDM sensitization. The results were very different when cockroach skin reactivity was analyzed: factors associated with HDM SPT were not associated with cockroach SPT as has been reported before[10]. In our study, only having farmer parents increased the risk of being cockroach SPT positive. We found no association between skin sensitization to these two aeroallergens and any of the potential risk factors measured when we considered the rural area possibly due to low number of SPT positive subjects and homogeneity regarding high helminth load or socio-economic status (SES) factors, such as, low paternal education was almost universal and all children were of farmer families.

Sensitization in terms of serum IgE to HDM and cockroach as well as total IgE showed entirely different patterns. In contrast to SPT, sIgE as well as total IgE were higher in the rural than in the semi-urban area. Helminth infections were associated with higher levels of total IgE in the semi-urban area and tended to increase sIgE to aeroallergens in both rural and semi-urban areas. The finding of enhanced IgE is in line with several previous studies that have demonstrated that total IgE increased with the presence of helminth infections[3,17,18].

Epidemiological studies have shown that high parental education, which is one of the indicators of high SES, is associated with atopy[9,19]. Our study also found that the prevalence of skin reactivity to HDM was influenced by paternal educational level. However, in our

Table 3. Association between specific or total IgE and potential risk factors for atopy in the semi-urban area[a]

		IgE to HDM[#]		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β [95% CI]	geometric mean [95% CI]	β [95% CI]	N	geometric mean [95% CI]	β [95% CI]
z-BMI (mean, SD)	574	-1.35 ± 1.08[b]	-0.02 [-0.08-0.03]	-1.35 ± 1.08b	-0.07 [-0.13--0.01]*	509	-1.26 ± 1.03[b]	-0.06 [-0.11--0.02]**
Paternal education								
Low	268	0.63 [0.52-0.77]	reference	1.55 [1.26-1.92]	reference	219	2231.6 [1877.8-2652.1]	reference
High	252	0.68 [0.55-0.85]	0.03 [-0.10-0.16]	1.10 [0.87-1.38]	-0.15 [-0.28--0.02]*	241	1644.5 [1382.7-1955.8]	-0.13 [-0.24--0.03]*
Maternal education	363	0.69 [0.58-0.82]	reference	1.65 [1.38-1.97]	reference	327	2201.7 [1917.7-2527.8]	reference
Low	199	0.60 [0.46-0.78]	-0.06 [-0.19-0.07]	0.94 [0.73-1.22]	-0.24 [-0.38--0.11]***	189	1382.2 [1138.7-1677.9]	-0.20 [-0.30--0.10]***
High								
Parental job	172	0.53 [0.41-0.69]	reference	0.89 [0.68-1.17]	reference	173	1442.4 [1192.3-1744.9]	reference
Non farmer	316	0.72 [0.60-0.88]	0.14 [0.00-0.28]	1.66 [1.36-2.02]	0.27 [0.13-0.42]***	262	2171.2 [1839.3-2562.9]	0.18 [0.07-0.29]**
Farmer								
House material	445	0.71 [0.61-0.83]	reference	1.52 [1.29-1.78]	reference	396	2018.9 [1780.8-2288.7]	reference
Bamboo / Wood	147	0.54 [0.40-0.72]	-0.12 [-0.26-0.02]	0.89 [0.65-1.22]	-0.23 [-0.38--0.09]**	144	1598.2 [1261.6-2024.6]	-0.10 [-0.21-0.01]
Stone	363	0.69 [0.58-0.82]	reference	1.65 [1.38-1.97]	reference	327	2201.7 [1917.7-2527.8]	reference
Water source								
Non piped	257	0.68 [0.55-0.84]	reference	1.49 [1.20-1.85]	reference	247	2016.3 [1706.3-2382.5]	reference
Piped	335	0.65 [0.54-0.79]	-0.02 [-0.14-0.11]	1.22 [1.01-1.48]	-0.09 [-0.21-0.04]	293	1801.8 [1550.2-2094.1]	-0.05 [-0.15-0.05]
Toilet								
No	58	0.63 [0.39-1.00]	reference	1.25 [0.74-2.09]	reference	52	2453.7 [1745.3-3449.7]	reference
Yes	534	0.67 [0.58-0.77]	0.03 [-0.18-0.23]	1.34 [1.15-1.56]	0.03 [-0.18-0.24]	488	1845.6 [1640.1-2076.7]	-0.12 [-0.29-0.04]

Table 3. Continued

		IgE to HDM[#]		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β [95% CI]	geometric mean [95% CI]	β [95% CI]	N	geometric mean [95% CI]	β [95% CI]
Floor material								
Mud	108	0.83 [0.60-1.16]	reference	2.05 [1.49-2.82]	reference	82	2343.0 [1753.7-3130.4]	reference
Cement / ceramic	485	0.63 [0.54-0.73]	-0.12 [-0.28-0.04]	1.21 [1.03-1.42]	-0.23 [-0.39--0.07]**	458	1826.5 [1618.5-2061.2]	-0.11 [-0.24-0.03]
Fuel								
Wood	506	0.65 [0.56-0.75]	reference	1.43 [1.23-1.67]	reference	459	1983.8 [1765.1-2229.5]	reference
Gas / kerosene	86	0.73 [0.47-1.12]	0.05 [-0.12-0.22]	0.87 [0.57-1.33]	-0.22 [-0.39--0.04]*	81	1471.8 [1047.8-2067.3]	-0.13 [-0.26-0.01]
Using sandals								
No	376	0.70 [0.59-0.84]	reference	1.46 [1.22-1.76]	reference	302	2027.8 [1751.5-2347.7]	reference
Yes	168	0.64 [0.49-0.82]	-0.04 [-0.18-0.09]	1.16 [0.89-1.51]	-0.10 [-0.24-0.04]	182	1771.6 [1450.3-2164.1]	-0.06 [-0.16-0.05]
N. americanus1								
Low load	173	0.62 [0.47-0.83]	reference	1.01 [0.76-1.35]	reference	98	1267.4 [954.3-1683.3]	reference
High load	133	0.68 [0.51-0.89]	0.04 [-0.14-0.21]	1.49 [1.13-1.96]	0.17 [-0.01-0.34]	90	2985.7 [2333.0-3820.9]	0.37 [0.21-0.54]***
A. lumbricoides1								
Low load	192	0.57 [0.44-0.74]	reference	1.00 [0.77-1.30]	reference	121	1535.5 [1199.9-1964.9]	reference
High load	114	0.80 [0.59-1.09]	0.15 [-0.03-0.33]	1.63 [1.21-2.18]	0.21 [0.03-0.39]*	67	2833.5 [2065.9-3886.1]	0.27 [0.09-0.44]**
T. trichiura2								
Negative	240	0.63 [0.51-0.79]	reference	1.17 [0.93-1.47]	reference	191	1865.2 [1539.0-2260.7]	reference
Positive	158	0.75 [0.56-1.00]	0.07 [-0.09-0.23]	1.47 [1.13-1.92]	0.10 [-0.05-0.26]	96	2043.4 [1590.5-2625.4]	0.04 [-0.10-0.18]

[a]association based on univariate logistic model. [b]Mean and standard deviation. The total population examined (N). [#]IgE to *Dermatophagoides pteronyssinus* (HDM). [1]diagnosed by PCR. [2]diagnosed by microscopy. β (beta): estimate regression coefficients. CI: Confidence intervals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4. Association between skin reactivity, specific IgE or total IgE and potential risk factors for atopy in the semi urban area[a]

	Skin prick test reactivity			
	HDM adj. OR [95% CI]	**Cockroach adj. OR [95% CI]**	**IgE to *B. germanica* adj. β (95% CI)**	**Total IgE adj. β (95% CI)**
General risk factors				
z-BMI			-0.04 (-0.14-0.06)	-0.01 (-0.10-0.07)
Paternal high education (reference: low)	1.82 [0.89-3.74]			
Maternal high education (reference: low)				-0.03 (-0.20-0.14)
Parental farmer (reference: non farmer)		1.53 [1.07-2.20]*	0.24 (0.04-0.44)*	
Piped water (reference: non piped)		0.84 [0.61-1.18]		
Specific parasite infection				
N. americanus (reference: low load)	0.46 [0.21-1.00]¶			0.33 (0.16-0.50)***
A. lumbricoides (reference: low load)			0.15 (-0.05-0.35)	0.22 (0.03-0.40)*

[a]Multivariate model adjusted with age and sex. β (beta): estimate regression coefficients. CI: Confidence intervals. *$P < 0.05$, ***$P < 0.001$, ¶$P < 0.1$

study paternal education disappeared as a significant predictive factor in multivariate analysis, while hookworm infection still remained as an independent protective factor on skin reactivity to HDM in the semi-urban area. Helminth infection has been shown in some studies to be inversely related with allergic skin sensitization[6,10]. As almost all of the population in rural area had intestinal helminth infection, this may explain the absence of an association between helminth infection and HDM reactivity in rural area.

In our semi-urban population we found that the prevalence of skin reactivity to cockroach was lower in children using piped water and higher in children from farmer parents. Piped water is a marker of higher SES, therefore would be expected to be associated with increased SPT. Two possibilities might explain our finding; (i) bacterial contamination of the water which could affect the immune system and thereby atopic sensitization[20], or (ii) piped water is associated with high SES and lower exposure to cockroach. However, in multivariate analysis only having farmer parents remained significantly associated with SPT reactivity to cockroach. The mechanism whereby being a child of farmer parents could increase risk of cockroach SPT positivity is not fully understood but might be related to increased exposure to this allergen.

In addition to having different profile of risk factors for SPT and IgE, another issue of interest is the finding that in the face of high IgE in rural area low SPT is seen. The reason for this discrepancy could not be addressed in this study but it might be due to the fact that helminth infection can potentially induce the production of false-positive serum IgE

through cross-reactivity between helminth and aeroallergens as proposed earlier[21]: this is in line with the findings that sensitization to cockroach may not be driven by true cockroach exposure but by cross-reactive carbohydrate determinants (CCDs) as demonstrated in a study from Ghana (Akkerdaas J *et al*, manuscript in preparation).

The limitation of this study was the cross sectional design in an area with no data on allergen exposure and we could not examine the relation between risk/protective factors with allergic outcomes in the rural area because it was very homogenous with respect to factors such as helminth infection load and socio-economic status. In addition, we did not measure sensitization to *Blomia tropicalis* which is an important mite allergen source in the tropics. Another limitation is that the use of questionnaires to assess information on the presence of allergic disorders such as asthma and risk factors could make the study to information bias. Misclassification of exposure may also have occurred in the present study since we did not evaluate past helminth infection. However, we used a sensitive technique to measure hookworm and *A. lumbricoides* infection load.

In conclusion, the higher prevalence of SPT to HDM in the semi-urban area might be due to high level of education and low helminth burden compared to the rural area. However, we could not explain the lower prevalence of cockroach allergies in the semi-urban area as we found that having a farming family and proxies for lower socioe-conomic status, if anything, were associated with increased risk of allergies to cockroach. Further studies are needed to evaluate the possible risk and protective factors in more detail as well as to check possible cross-reactivity between allergens and helminths. So far, our data provide useful information on environmental as well as socio-economic factors which can be considered by clinicians and researchers working on prevention, diagnosis and treatment of allergic disorders in low-to-middle income countries.

REFERENCES

1 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. Lancet 1998;351:1225-32.

2 Addo Yobo EO, Custovic A, et al. Exercise induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. Thorax 1997;52:161-5.

3 Perzanowski MS, Ng'ang'a LW, et al. Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. J Pediatr 2002;140: 582-8.

4 von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. J Allergy Clin Immunol 2002;109:S525-32.

5 Borchers AT, Keen CL, et al. Hope for the hygiene hypothesis: when the dirt hits the fan. J Asthma 2005;42:225-47.

6 Feary J, Britton J, et al. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66:569-78.

7 van den Biggelaar AH, Lopuhaa C, et al. The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. Int Arch Allergy Immunol 2001;126:231-8.

8 Supali T, Djuardi Y, et al. Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. Int Arch Allergy Immunol 2010;153:388-94.

9 Cooper PJ, Chico ME, et al. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004;34:845-52.

10 Flohr C, Tuyen LN, et al. Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: A cross-sectional study. J Allergy Clin Immunol 2006;118:1305-11.

11 Bethony J, Brooker S, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521-32.

12 Weinmayr G, Weiland SK, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2007;176:565-74.

13 Wiria AE, Prasetyani MA, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2010;10:77.

14 Hamid F, Wiria AE, et al. A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2011;11: 83.

15 WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/ height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization 2006;312.

16 Nyan OA, Walraven GE, et al. Atopy, intestinal helminth infection and total serum IgE in rural and urban adult Gambian communities. Clin Exp Allergy 2001;31:1672-8.

17 Blackwell AD, Gurven MD, et al. Evidence for a peak shift in a humoral response to helminths: age profiles of IgE in the Shuar of Ecuador, the Tsimane of Bolivia, and the U.S. NHANES. PLoS Negl Trop Dis 2011;5:e1218.

18 Flohrs K, Bruske I, et al. Temporal changes in total serum immunoglobulin E levels in East German children and the effect of potential predictors. Int Arch Allergy Immunol 2012;158:27-34.

19 Forastiere F, Agabiti N, et al. Socioeconomic status, number of siblings, and respiratory infections in early life as determinants of atopy in children. Epidemiology 1997;8:566-70.

20 von Hertzen L, Laatikainen T, et al. Microbial content of drinking water in Finnish and Russian Karelia - implications for atopy prevalence. Allergy 2007;62:288-92.

21 Santos AB, Rocha GM, et al. Cross-reactive IgE antibody responses to tropomyosins from Ascaris lumbricoides and cockroach. J Allergy Clin Immunol 2008;121:1040-6.

22 Baratawidjaja IR, Baratawidjaja PP, et al. (1999) Prevalence of allergic sensitization to regional inhalants among allergic patients in Jakarta, Indonesia. Asian Pac J Allergy Immunol 1999;17:9-12.

SUPPLEMENTARY TABLES

Table S1. Association between skin reactivity to HDM or cockroach and potential risk factors for atopy in the rural area[a]

		HDM		Cockroach	
	N	n (%)	OR [95% CI]	n (%)	OR [95% CI]
z-BMI (mean, SD)	447	-1.68 ± 1.14b	1.11 [0.83-1.49]	-1.68 ± 1.14b	0.86 [0.70-1.06]
Paternal education					
Low	317	26 (8.2)	reference	59 (18.6)	reference
High	81	11 (13.6)	1.76 [0.83-3.73]	17 (21.0)	1.16 [0.63-2.13]
Maternal education					
Low	364	32 (8.8)	reference	62 (17.0)	reference
High	74	6 (8.1)	0.92 [0.37-2.27]	18 (24.3)	1.57 [0.86-2.84]
Parental job					
Non farmer	20	0		2 (10.0)	reference
Farmer	367	37 (10.1)	-	72 (19.6)	2.20 [0.50-9.68]
House material					
Bamboo / Wood	410	36 (8.8)	reference	76 (18.5)	reference
Stone	37	2 (5.4)	0.59 [0.14-2.57]	6 (16.2)	0.85 [0.34-2.11]
Water source					
Non piped	447	38 (8.5)		82 (18.3)	
Piped	0	0	-	0	-
Toilet					
No	199	19 (9.5)	reference	36 (18.1)	reference
Yes	248	19 (7.7)	0.79 [0.40-1.53]	46 (18.5)	1.03 [0.64-1.67]
Floor material					
Mud	340	28 (8.2)	reference	60 (17.6)	reference
Cement / ceramic	107	10 (9.3)	1.15 [0.54-2.45]	22 (20.6)	1.21 [0.70-2.08]
Fuel					
Wood	430	36 (8.4)	reference	81 (18.8)	reference
Gas / kerosene	17	2 (11.8)	1.46 [0.32-6.63]	1 (5.9)	0.27 [0.04-2.06]
Using sandals					
No	425	37 (8.7)	reference	76 (17.9)	reference
Yes	22	1 (4.5)	0.50 [0.07-3.82]	6 (27.3)	1.72 [0.65-4.54]
N. americanus[1]					
Low load	41	5 (12.2)	reference	8 (19.5)	reference
High load	149	13 (8.7)	0.69 [0.23-2.06]	31 (20.8)	1.08 [0.46-2.58]
A. lumbricoides[1]					
Low load	161	16 (9.9)	reference	35 (21.7)	reference
High load	29	2 (6.9)	0.67 [0.15-3.09]	4 (13.8)	0.58 [0.19-1.77]
T. trichiura[2]					
Negative	170	17 (10.0)	reference	30 (17.6)	reference
Positive	52	4 (7.7)	0.75 [0.24-2.34]	12 (23.1)	1.40 [0.66-2.98]

[a]association based on univariate logistic model. [b]Mean and standard deviation. [1]diagnosed by PCR. [2]diagnosed by microscopy. The number of positives (n) of the total population examined (N). OR: Odds ratio, CI: Confidence intervals. *$P < 0.05$

Table S2. Association between specific or total IgE and potential risk factors for atopy in the rural area[a]

		IgE to HDM[#]		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β [95% CI]	geometric mean [95% CI]	β [95% CI]	N	geometric mean [95% CI]	β [95% CI]
z-BMI (mean, SD)	290	-1.75 ± 1.15[b]	-0.02 [-0.08-0.05]	-1.75 ± 1.15b	0.02 [-0.05-0.09]	311	-1.73 ±1.15[b]	0.02 [-0.03-0.07]
Paternal education								
Low	200	1.12 [0.91-1.38]	reference	1.73 [1.38-2.18]	reference	238	4178.1 [3590.0-4862.4]	reference
High	56	1.13 [0.71-1.78]	0.00 [-0.20-0.20]	1.39 [0.90-2.16]	-0.10 [-0.31-0.12]	66	4040.8 [2939.0-5555.5]	-0.01 [-0.16-0.13]
Maternal education								
Low	236	1.09 [0.91-1.31]	reference	1.60 [1.30-1.97]	reference	280	4400.4 [3831.5-5053.7]	reference
High	50	1.27 [0.76-2.13]	0.07 [-0.13-0.26]	1.89 [1.20-2.98]	0.07 [-0.14-0.29]	55	3116.9 [2299.5-4224.8]	-0.15 [-0.30-0.00]*
Parental job								
Non farmer	15	0.68 [0.30-1.55]	reference	0.90 [0.44-1.81]	reference	15	3276.5 [2215.8-4845.1]	reference
Farmer	233	1.15 [0.95-1.41]	0.23 [-0.12-0.58]	1.71 [1.38-2.13]	0.28 [-0.09-0.66]	281	4263.9 [3688.2-4929.3]	0.11 [-0.16-0.39]
House material								
Bamboo / Wood	265	1.13 [0.94-1.36]	reference	1.70 [1.39-2.06]	ref	309	4088.4 [3596.0-4648.2]	reference
Stone	27	1.05 [0.66-1.68]	-0.03 [-0.29-0.22]	1.35 [0.73-2.52]	-0.10 [-0.38-0.18]	31	4705.3 [2804.6-7893.9]	0.06 [-0.13-0.25]
Water source								
Non piped	292	1.13 [0.95-1.34]		1.66 [1.38-2.00]		340	4141.1 [3654.7-4692.3]	
Piped	0	0	-	0	-	0	0	-
Toilet								
No	115	0.97 [0.74-1.28]	reference	1.52 [1.13-2.04]	reference	141	4071.4 [3347.4-4951.9]	reference
Yes	177	1.24 [0.99-1.54]	0.11 [-0.05-0.26]	1.76 [1.38-2.24]	0.06 [-0.10-0.23]	199	4191.3 [3558.5-4936.6]	0.01 [-0.10-0.12]

Table S2. Association between specific or total IgE and potential risk factors for atopy in the rural area[a]

		IgE to HDM[#]		IgE to *B. germanica*			Total IgE	
	N	geometric mean [95% CI]	β [95% CI]	geometric mean [95% CI]	β [95% CI]	N	geometric mean [95% CI]	β [95% CI]
Floor material								
Mud	222	1.03 [0.84-1.26]	reference	1.60 [1.28-2.00]	reference	256	3906.7 [3398.4-4490.9]	reference
Cement / ceramic	70	1.48 [1.07-2.04]	0.16 [-0.02-0.33]	1.87 [1.36-2.58]	0.07 [-0.12-0.26]	84	4946.1 [3751.9-6520.3]	0.10 [-0.02-0.23]
Fuel								
Wood	278	1.13 [0.95-1.35]	reference	1.70 [1.40-2.06]	reference	328	4251.4 [3743.5-4828.2]	reference
Gas / kerosene	14	1.03 [0.55-1.94]	-0.04 [-0.39-0.31]	1.08 [0.55-2.09]	-0.20 [-0.58-0.18]	12	2019.0 [1099.1-3708.8]	-0.32 [-0.62--0.03]*
Using sandals								
No	281	1.13 [0.95-1.34]	reference	1.63 [1.35-1.97]	reference	298	4248.9 [3728.1-4842.4]	reference
Yes	9	1.22 [0.23-6.64]	0.04 [-0.40-0.47]	3.71 [1.07-12.83]	0.36 [-0.11-0.82]	13	3981.0 [1694.4-9353.3]	-0.03 [-0.31-0.25]
N. americanus1								
Low load	36	0.62 [0.41-0.94]	reference	0.97 [0.55-1.74]	reference	29	3243.7 [2147.5-4899.5]	reference
High load	125	1.10 [0.84-1.45]	0.25 [0.01-0.49]*	1.76 [1.31-2.36]	0.26 [-0.02-0.53]	117	4476.4 [3626.0-5526.2]	0.14 [-0.06-0.34]
A. lumbricoides1								
Low load	139	0.99 [0.76-1.28]	reference	1.59 [1.19-2.12]	reference	128	4347.4 [3537.0-5343.4]	reference
High load	22	0.87 [0.52-1.46]	-0.05 [-0.35-0.24]	1.27 [0.63-2.53]	-0.10 [-0.43-0.24]	18	3279.9 [2199.5-4891.0]	-0.12 [-0.37-0.12]
T. trichiura2								
Negative	151	1.03 [0.82-1.31]	reference	1.58 [1.22-2.05]	reference	137	3989.2 [3275.0-4859.1]	reference
Positive	42	1.25 [0.77-2.03]	0.08 [-0.14-0.30]	1.89 [1.08-3.28]	0.08 [-0.17-0.32]	45	5812.0 [3917.7-8622.1]	0.16 [-0.01-0.34]

[a]association based on univariate logistic model. [b]Mean and standard deviation. The total population examined (N). [#]IgE to *Dermatophagoides pteronyssinus* (HDM). [1]diagnosed by PCR. [2]diagnosed by microscopy. β (beta): estimate regression coefficients. CI: Confidence intervals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table S3. Association between specific IgE or total IgE and potential risk factors for atopy in the rural area[a]

	IgE to HDM[#] adjusted β (95% CI)	**Total IgE adjusted β (95% CI)**
General risk factors		
Fuel kerosene/gas (reference: wood)		-0.31 (-0.60- -0.30)*
Specific parasite infection		
N. americanus (reference: low load)	0.27 (0.03-0.51)*	

[a]Multivariate model adjusted with age and sex. [#]IgE to *Dermatophagoides pteronyssinus* (HDM).
β (beta): estimate regression coefficients. CI: Confidence intervals. *$P < 0.05$.

THE EFFECT OF THREE-MONTHLY ALBENDAZOLE TREATMENT ON MALARIAL PARASITEMIA AND ALLERGY: A HOUSEHOLD-BASED CLUSTER-RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

Aprilianto E Wiria[1,2,*], Firdaus Hamid[2,3,*], Linda J Wammes[2,*], Maria MM Kaisar[1,2], Linda May[2], Margaretta A Prasetyani[1,2], Sitti Wahyuni[4], Yenny Djuardi[1,2], Iwan Ariawan[5], Heri Wibowo[1], Bertrand Lell[6,7], Robert Sauerwein[8], Gary T Brice[9], Inge Sutanto[1], Lisette van Lieshout[2], Anton JM de Craen[10], Ronald van Ree[11], Jaco J Verweij[2], Roula Tsonaka[12], Jeanine J Houwing-Duistermaat[12], Adrian JF Luty[8], Erliyani Sartono[2], Taniawati Supali[1], Maria Yazdanbakhsh[2]

1. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
4. Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
5. Department of Biostatistics, School of Public Health, University of Indonesia, Jakarta, Indonesia
6. Medical Research Unit, Albert Schweitzer Hospital, Lambaréné, Gabon
7. Department of Parasitology, Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany
8. Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
9. Naval Medical Research Unit 2, Jakarta, Indonesia
10. Department of Gerontology and Geriatrics, Leiden University Medical Center, Leiden, The Netherlands
11. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
12. Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

**these authors contributed equally*

PLoS ONE 2013;8: e578995

ABSTRACT

Background Helminth infections are proposed to have immunomodulatory activities affecting health outcomes either detrimentally or beneficially. We evaluated the effects of albendazole treatment, every three months for 21 months, on STH, malarial parasitemia and allergy.

Methods A household-based cluster-randomized, double-blind, placebo-controlled trial was conducted in an area in Indonesia endemic for STH. Using computer-aided block randomization, 481 households (2022 subjects) and 473 households (1982 subjects) were assigned to receive placebo and albendazole, respectively, every three months. The treatment code was concealed from trial investigators and participants. Malarial parasitemia and malaria-like symptoms were assessed in participants older than four years of age while skin prick test (SPT) to allergens as well as reported symptoms of allergy in children aged 5–15 years. The general impact of treatment on STH prevalence and body mass index (BMI) was evaluated. Primary outcomes were prevalence of malarial parasitemia and SPT to any allergen. Analysis was by intention to treat.

Results At 9 and 21 months post-treatment 80.8% and 80.1% of the study subjects were retained, respectively. The intensive treatment regiment resulted in a reduction in the prevalence of STH by 48% in albendazole and 9% in placebo group. Albendazole treatment led to a transient increase in malarial parasitemia at 6 months post treatment (OR 4.16(1.35–12.80)) and no statistically significant increase in SPT reactivity (OR 1.18 (0.74–1.86) at 9 months or 1.37 (0.93–2.01) 21 months). No effect of anthelminthic treatment was found on BMI, reported malaria-like- and allergy symptoms. No adverse effects were reported.

Conclusions The study indicates that intensive community treatment of 3 monthly albendazole administration for 21 months over two years leads to a reduction in STH. This degree of reduction appears safe without any increased risk of malaria or allergies.

Trial Registration Controlled-Trials.com ISRCTN83830814

INTRODUCTION

Soil transmitted helminths (STH) (hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*) establish chronic infections in a large proportion of the world population[1]. Major intervention programs using mass drug administration (MDA) to control STH have been launched[2]. However, STH infections seem to persist in the targeted populations raising concern over the development of drug resistance[3]. It is therefore important to conduct well-designed studies that allow evidence-based decisions to be made to maximize effective STH control toward elimination.

While there is no doubt that STH are associated with morbidities in billions of people worldwide, there is also increasing awareness that helminth infections might, like bacterial commensals, play an important role in shaping human health[4]. Helminths may contribute to immunologic and physiologic homeostasis. The immune system is thought to have evolved to operate optimally in the face of helminth-induced immune regulation, and that any disturbance of this long evolutionary co-existence between humans and helminth parasites might be associated with the emergence of pathological conditions[5] possibly involving outcomes of exposure to other pathogens or the development of inflammatory diseases.

In many parts of the world helminths and malarial parasites are co-endemic raising the question of what impact helminth infections may have on the plasmodial parasites that cause malaria. The results have been conflicting in this regard. In some studies a positive association has been reported between helminths and malarial parasitemia while in others, this has been refuted or in yet others a negative association has been shown between helminths and the severity of the clinical outcomes of malaria (reviewed by Nacher)[6].

An increase in the prevalence of allergies has been reported worldwide, in particular in the urban areas of low- to middle-income countries[7]. Although majority of cross-sectional studies have reported inverse associations between helminth infections and allergies[8,9], two randomized trials with albendazole, have shown conflicting results. One in Ecuador, based on school randomization, reported no change in either SPT reactivity to allergens or allergic symptoms after one year of albendazole treatment[10] while another in Vietnam, in which the randomization unit was individual schoolchildren, showed increased SPT reactivity after one year of albendazole treatment, but consistent with the Ecuadorean study, clinical allergy did not change significantly[11]. It has been suggested that anthelminthic treatment of longer duration might be needed to reveal the modulatory effect of helminths[12,13].

In the light of global deworming initiatives, it is important to assess the effectiveness of and to monitor the risks associated with anthelminthic treatment regiments. There is as yet no report of a household-based cluster-randomized double-blind placebo-controlled trial of repeated anthelminthic administration in a community that would be expected to more effectively reduce transmission of STH by decreasing household cross-contamination.

In an area where STH and malaria are co-endemic on Flores Island, Indonesia, we conducted a household cluster-randomized trial of three-monthly albendazole treatment over a two year study period in a whole community to assess benefits and risks associated with this anthelmintic treatment. Specifically we assessed its impact on STH, malarial parasitemia and allergy.

METHODS

Study population and design

This trial was conducted in two villages in the Ende District of Flores Island, Indonesia (Appendix S1, p2) as described in detail elsewhere[14,15]. The treatment was based on household and given to all household members except those less than two years old or pregnant (the Indonesian national program guideline). Directly observed treatment was given three monthly during the trial period (June 2008 to July 2010, with treatment starting in Sept 2008). The primary outcomes were prevalence of malarial parasitemia and SPT reactivity to allergens. Additional outcomes were treatment effect on STH and BMI as well as malaria-like and allergy symptoms.

We measured malaria outcomes in Nangapanda only. Malaria was not endemic in Anaranda. Artemisinin-combination therapy (ACT) treatment and treated bed net distribution were not implemented during our study period[16,17].

Allergy outcomes were measured, in both villages, in school-age children (5–15 years old) as this group is particularly at risk of developing allergy and asthma[18] and is the target population of global deworming programs.

The study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia (ethical clearance ref: 194/PT02.FK/Etik/2006) and filed by the Committee of Medical Ethics of the Leiden University Medical Center. The trial was registered as clinical trial (Ref: ISRCTN83830814). Prior to the study, written informed consent was obtained from participants or from parents/guardians of children. The study is reported in accordance with the CONSORT guidelines for cluster-randomized studies.

Randomization and masking

The population was randomized by Iwan Ariawan (IA) using computer aided block randomization at household level utilising Random Allocation software to receive albendazole (single dose of 400 mg) or a matching placebo (both tablets from PT Indofarma Pharmaceutical, Bandung, Indonesia). The treatment code was concealed from trial investigators and participants. The un-blinding of treatment codes occurred after all laboratory results had been entered into the database (August 2011).

Procedures

Trained community workers measured fever, administered monthly malaria-like symptoms questionnaire which was based on WHO definitions[19] and took finger-prick blood for the three-monthly malarial parasitemia survey. Subjects with fever (≥37.5°C) or additional malaria-like symptoms (headache, fatigue and nausea) at the time of visits were referred to the local primary health centre (puskesmas). Thick and thin Giemsa-stained blood smears were read at University of Indonesia. At baseline, 9 months and 21 months after the first round of treatment blood was collected for PCR-based detection of *Plasmodium spp*. (supplementary methods), a method that is more sensitive than microscopy[20].

Regarding allergy outcomes, skin prick tests (SPT) with allergens were performed on school-age children in Nangapanda and Anaranda and clinical symptoms of allergy were recorded. House dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*; kindly provided by Paul van Rijn from HAL Allergy Laboratories, Leiden, The Netherlands) and cockroach (*Blattella germanica*; Lofarma, Milan, Italy) were used for SPT which was considered positive with 3 mm cut off[14]. The SPT was performed by one investigator. IgE with specificity for aeroallergens (*D. pteronyssinus* and *B. germanica*) was measured in plasma using an ImmunoCAP 250 system (Phadia, Uppsala, Sweden) following the manufacturer's instructions. All measurements were conducted in one laboratory in the Netherlands. Symptoms of asthma and atopic dermatitis were recorded using a modified visually-assisted version of the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire as reported before[14].

Yearly stool samples were collected on a voluntary basis. *Trichuris* was detected by microscopy and a multiplex real-time PCR was used for detection of hookworms (*Ancylostoma duodenale*, *Necator americanus*), *Ascaris lumbricoides*, and *Strongyloides stercoralis* DNA as detailed before[15] (supplementary methods). Very few subjects were infected with *S. stercoralis* and therefore this infection was not included in analyses.

Body weight and height were measured using the National Heart Lung and Blood Institute practical guidelines (scale and microtoise from SECA GmBH & Co, Hamburg, Germany).

Power calculation

Sample size estimation was based on the expected change in primary outcomes taking into account a power of 90% and a significance level of <0.05 with a loss to follow-up of 20%. Based on previous observations we expected to find a decrease in malarial parasitemia prevalence and an increase in SPT reactivity after anthelminthic treatment. Based on a prevalence of about 10% and a risk ratio (RR) of 0.60 we aimed to include 2412 people in the malaria assessments. In a pilot study we found SPT to *D. pteronyssinus* to be around 15%, and expected that due to treatment the prevalence would increase. In order to find a RR of 1.5 we aimed to include at least 1418 children.

Statistical analyses

For children ≤19 years, BMI age-standardized z-scores were calculated according to WHO references[21]. The IgE data were log-transformed to obtain normally distributed variable. To assess treatment effects generalized linear mixed models were used which provide a flexible and powerful tool to derive valid inference while capturing the data correlations induced by clustering within households and repeated evaluations in time of the same subject. Parameter estimates for treatment effects at 9 and 21 months and 95% confidence intervals are reported. The reported *P*-values were obtained using likelihood ratio tests by comparing the model with and without the treatment effect. Unless stated otherwise, all outcomes were adjusted for area (the two study villages in Ende District: Nangapanda or Anaranda) as covariate in the model. For the continuous outcomes linear mixed-effects models[22] were used with three random effects,

namely to model clustering within households, a random household specific intercept was used and to model correlation within subjects a random subject specific intercept and slope were used. For the binary outcomes a logistic model was used with random household effects and random subject effects. All models were fitted using the lme4 package (supplementary appendix, p6-7)[23]. For each fever and additional malaria-like symptoms, total number of events and person months are computed for each treatment arm. Hazard ratios for effect of treatment were calculated with Cox regression with robust SE to allow for within-household clustering (STATA 11).

RESULTS

At baseline, 954 households with 4004 subjects were registered. Randomization of households resulted in 1982 people assigned to albendazole treatment and 2022 people to placebo (473 and 481 houses respectively).

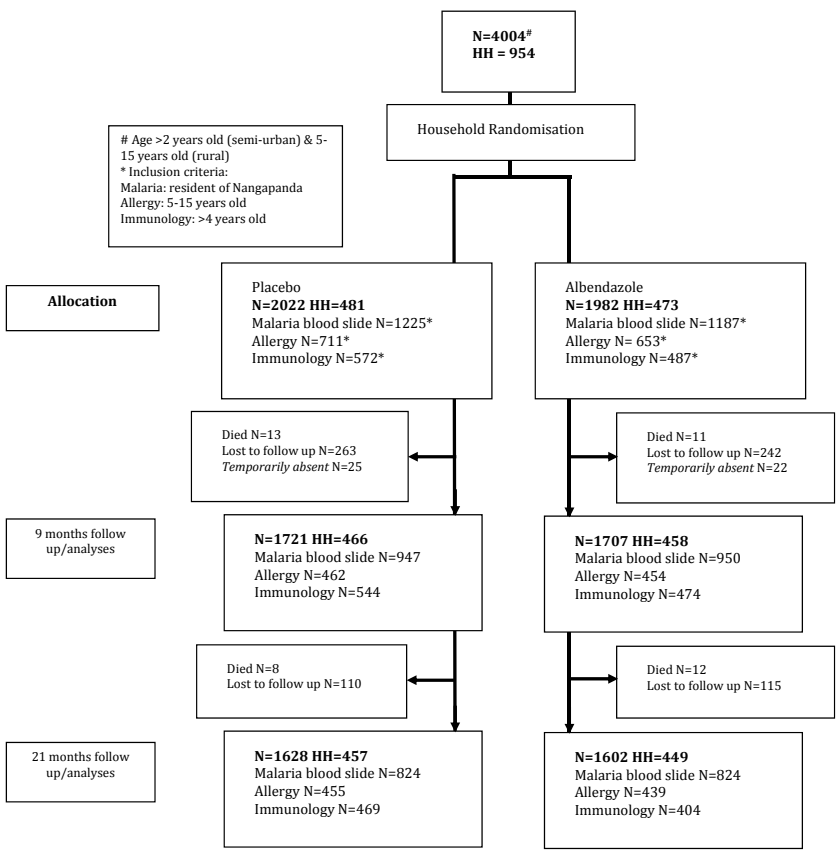


Figure 1. Trial Profile. *HH: Household. Lost to follow up implies that the participants have no data from this time point onward. Temporarily absent implies that the participants have no data at this time point but have data available at other time point*

At baseline 87·3% of the individuals were infected with one or more helminth species. The baseline characteristics were similar between the treatment arms (Table 1). The overall trial profile is shown in Figure 1, and Figure S1A, S1B, S1C in supplementary figures separately for malaria, allergy and helminth outcomes. The analysis was intention-to-treat and involved all participants as assigned randomly at the start of the trial. During the study, in the albendazole arm 61 people moved to a house that was assigned to placebo while in the placebo arm 62 people moved to a house that was assigned to albendazole. The 44

Table 1. Baseline characteristic study population

	N	Placebo	N	Albendazole
Age (mean in years, SD)	2022	25.7 (18.7)	1982	25.8 (18.7)
Sex (female, n, %)	2022	1090 (53.9)	1982	1042 (52.6)
Area (rural, n, %)	2022	260 (12.9)	1982	253 (12.8)
BMI > 19 years old (mean, SD)	575	22.3 (4.0)	582	21.8 (3.6)
Z score of BMI ≤ 19 years old (mean, SD)	427	-1.20 (1.2)	386	-1.37 (1.3)
Parasite infection (n, %)				
Helminth *(*any *spp)*	655	571 (87.2)	609	533 (87.5)
Hookworm[1]	683	509 (74.5)	629	486 (77.3)
N. americanus[1]	683	503 (73.7)	629	481 (76.5)
A. duodenale[1]	683	44 (6.4)	629	41 (6.5)
A. lumbricoides[1]	683	238 (34.9)	629	209 (33.2)
S. stercoralis[1]	683	7 (1.0)	629	18 (2.9)
T. trichiura[2]	953	258 (27.1)	852	237 (27.8)
Malarial parasitemia *(*any *spp)*[2]	1225	60 (4.9)	1187	52 (4.4)
P. falciparum	1225	32 (2.6)	1187	28 (2.4)
P. vivax	1225	26 (2.1)	1187	18 (1.5)
P. malariae	1225	2 (0.2)	1187	7 (0.6)
Malarial parasitemia *(*any *spp)*[1]	772	195 (25.3)	739	200 (27.1)
P. falciparum	772	106 (13.7)	739	112 (15.2)
P. vivax	772	102 (13.2)	739	93 (12.6)
P. malariae	772	10 (1.3)	739	18 (2.4)
Skin prick reactivity (n, %)				
Any allergen	711	190 (26.7)	653	163 (25.0)
House dust mite	711	88 (12.4)	653	75 (11.5)
Cockroach	711	163 (22.9)	653	140 (21.4)
Specific IgE, kU_A/L (median, IQR)				
House dust mite	452	0.8 (0.3-2.6)	431	0.8 (0.2-2.4)
Cockroach	452	1.5 (0.4-5.7)	431	1.9 (0.5-5.0)

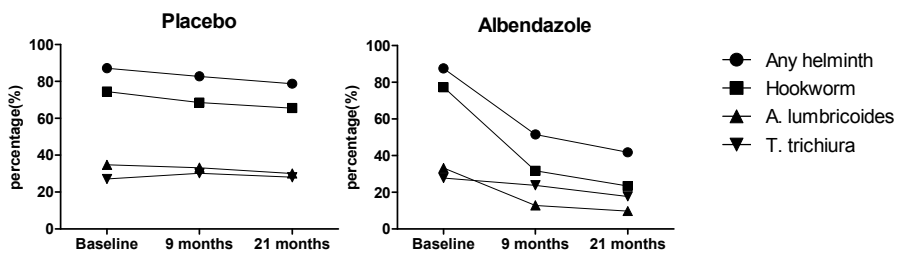
[1]diagnosed by PCR; [2]diagnosed by microscopy. The number of positives (n) of the total population examined (N)

subjects who died during the trial, included 4 people below the age of 20, 3 between 20 and 40 and the rest above 40 years of age, and were equally distributed between the treatment arms. At 9 months post-treatment full compliance was 77.8% for albendazole treatment and 78.0% for placebo. This was 63.1% and 62.5% respectively at 21 months.

This intensive treatment with albendazole resulted in a reduction but not elimination of STH. There was a decrease both after 9 (OR (95% CI) = 0.07 (0.04–0.11) and 21 months (0.05 (0.03–0.08)) of treatment ($P < 0.0001$). Albendazole had the largest effect on hookworm followed by *Ascaris* while the effect on *Trichuris* was less pronounced (Figure 2A and Table S1 in supplementary tables). Treatment also led to statistically significant reduction in the intensity of hookworm and *Ascaris* infection as determined by PCR (Figure 2B).

The fact that the stool sampling was on a voluntary basis could have created a selection bias. Analyzing baseline characteristics of subjects providing stool samples and those who did not at 9 months follow up, showed no differences in helminth prevalence, age and sex.

A. Percentage of helminth infected subjects in placebo and albendazole treatment arms



B. Effect of albendazole treatment on reduction in the intensity as well as percentage of subjects positive for hookworm and Ascaris infection as determined by PCR

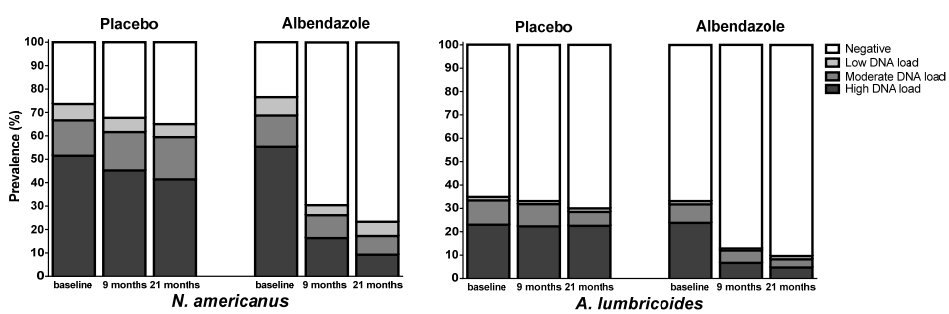


Figure 2. Effect of treatment on helminth prevalence and intensity. *A) Percentage of helminth infected subjects in placebo and albendazole treatment arms. The presence of hookworms (by PCR), Ascaris lumbricoides (by PCR) and Trichuris trichiura (by microscopy) or any of these helminth infections in subjects who provided stool samples at baseline, 9 and 21 months post treatment (numbers are given in Table S1A). B) Effect of albendazole treatment on reduction in the intensity as well as percentage of subjects positive for hookworm and Ascaris infection as determined by PCR. Negative is when no helminth specific DNA was found. Positive Ct- values were grouped into three categories: Ct<30.0, 30.0≤Ct<35.0 and ≥35.0 representing a high, moderate and low DNA load, respectively.*

Although at 21 months post treatment, sex and helminth prevalence were not different, age was slightly but significantly higher in subjects who provided stool samples, with a mean age in years (SD) = 29.9 (20.4) vs 24.3 (17.5), $P = 0.006$).

The overall percentage of subjects with malarial parasitemia, irrespective of treatment arm, decreased over the trial period (Table 2). However, when the data were modelled to assess the effect of albendazole treatment over time, there was a significant ($P = 0.0064$) increase, which might result from the transient four-fold increased risk of malarial parasitemia (OR 4.16 (1.35–12.80)) (Table 3) at 6 months after initiation of treatment (after 2 doses of albendazole).

Table 2. Effect of three-monthly albendazole treatment on malaria outcomes: percentage of subject with malarial parasitemia.

	P. falciparum		*P. vivax*		*P. malariae*	
	Placebo n/N (%)	**Albendazole n/N (%)**	**Placebo n/N (%)**	**Albendazole n/N (%)**	**Placebo n/N (%)**	**Albendazole n/N (%)**
Malarial parasitemia by microscopy						
0 month	32/1225 (2.6)	28/1187 (2.4)	26/1225 (2.1)	18/1187 (1.5)	2/1225 (0.2)	7/1187 (0.6)
3 months	41/897 (4.6)	46/910 (5.1)	17/897 (1.9)	22/910 (2.4)	1/897 (0.1)	6/910 (0.7)
6 months	8/815 (1.0)	20/794 (2.5)	4/815 (0.5)	9/794 (1.1)	0	0
9 months	14/947 (1.5)	7/950 (0.7)	4/947 (0.4)	5/950 (0.5)	1/947 (0.1)	1/950 (0.1)
12 months	9/834 (1.1)	9/813 (1.1)	4/834 (0.5)	2/813 (0.2)	0	0
15 months	14/773 (1.8)	13/772 (1.7)	3/773 (0.4)	4/772 (0.5)	1/773 (0.1)	3/772 (0.4)
18 months	3/815 (0.4)	10/803 (1.2)	1/815 (0.1)	1/803 (0.1)	1/815 (0.1)	1/803 (0.1)
21 months	6/824 (0.7)	11/824 (1.3)	6/824 (0.7)	0	3/824 (0.4)	1/824 (0.1)
Malarial parasitemia by PCR						
0 month	106/772 (13.7)	112/739 (15.2)	102/772 (13.2)	93/739 (12.6)	10/772 (1.3)	18/739 (2.4)
9 months	35/656 (5.3)	56/627 (8.9)	56/656 (8.5)	50/627 (8.0)	7/656 (1.1)	9/627 (1.4)
21 months	21/584 (3.6)	31/553 (5.6)	24/584 (4.1)	27/553 (4.9)	10/584 (1.7)	5/553 (0.9)

The number of positives (n) of the total population examined (N).

The effect of anthelminthic treatment was assessed in those younger than 15 years of age who would be the prime target of the global deworming programs. The transient increase in parasitemia was only seen in the older (>15 years) age group (Figure 3).

Malarial parasites were also assessed by PCR, at 9 and 21 months after initiation of treatment and revealed that albendazole had no effect when all *Plasmodium* species were considered together, but when analyzed separately there was a significant increase in the percentage of subjects positive for *P. falciparum* at 9 months post-treatment (Table 4). There was no difference in the incidence of fever and additional malaria-like symptoms between the two treatment arms (Table S2).

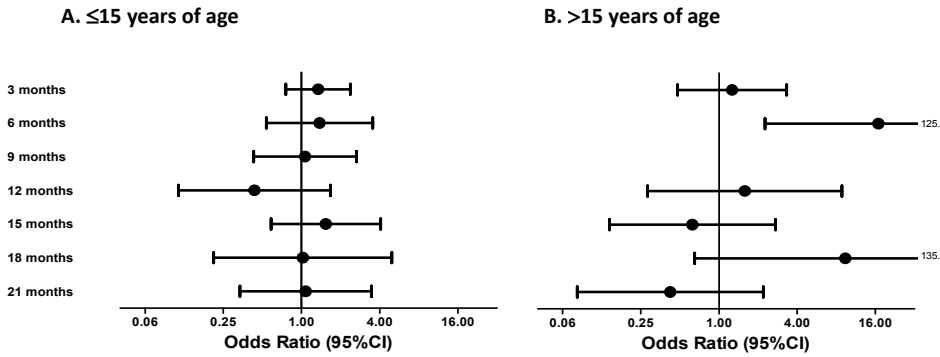


Figure 3. Effect of albendazole treatment on malarial parasitemia based on two age categories. *Malarial parasitemia A) ≤15 and B) >15 years of age. The risk of malarial parasitemia after albendazole treatment compared to placebo is shown as odds ratio with 95% CI. The reference line is set at 1, indicating that symbols at the right of this line represent an increased risk, while symbols at the left of the line would predict decreased risk of malarial parasitemia. Note: at 9 month time point in those >15 years of age, the OR is ∞*

Table 3. Effect of three-monthly albendazole treatment on malaria outcome: Malarial parasitemia by microscopy

	Placebo n/N (%)	Albendazole n/N (%)	OR (95%CI)
Malarial parasitemia (any *spp*)			
3 months	59/897 (6.6)	72/910 (7.9)	1.54 (0.75-3.16)
6 months	12/815 (1.5)	29/794 (3.7)	4.16 (1.35-12.80)
9 months	19/947 (2.0)	13/950 (1.4)	0.57 (0.16-2.04)
12 months	13/834 (1.6)	10/813 (1.2)	0.62 (0.12-3.15)
15 months	18/773 (2.3)	20/772 (2.6)	1.17 (0.18-7.65)
18 months	5/815 (0.6)	12/803 (1.5)	1.84 (0.12-29.03)
21 months	15/824 (1.8)	12/824 (1.5)	0.26 (0.01-6.59)

The number of positives (n) of the total population examined (N). Odds ratio and 95% confidence interval are based on mixed effects logistic regression models. OR's and 95% CI are shown for the separate time points on malarial parasitemia. The *P*-value is generated from the modeled data for the combined effect of albendazole treatment over time, which is significant ($P = 0.0064$) and might result from the effect of 6 months post treatment time point

The proportion of subjects with SPT reactivity was 353/1364 (25.9%) at baseline. Albendazole treatment had no statistically significant effect on SPT to any allergen (Table 5), but it was noted that there was an incremental increase in the risk of being SPT positive to any allergen at 9 months and 21 months post initiation of treatment. Moreover, additional analysis on allergens separately, showed a significantly higher SPT to cockroach at 21 months after treatment (OR 1.63 (1.07–2.50)) (Table 6). The levels of IgE to allergens showed that albendazole treatment had no effect on sensitization (Table 6). No effect of treatment was seen on symptoms of asthma or atopic dermatitis (Table S3).

Table 4. Effect of three-monthly albendazole treatment on malaria outcome: Malarial parasitemia by PCR

	Placebo n/N (%)	Albendazole n/N (%)	OR (95% CI)
Malaria (any spp)			
9 months	95/656 (14.5)	103/627 (16.4)	1.13 (0.77-1.64)
21 months	53/584 (9.1)	59/553 (10.7)	1.09 (0.68-1.76)
P. falciparum			
9 months	35/656 (5.3)	56/627 (8.9)	2.82 (1.29-6.15)
21 months	21/584 (3.6)	31/553 (5.6)	1.63 (0.63-4.22)
P. vivax			
9 months	56/656 (8.5)	50/627 (8.0)	0.84 (0.41-1.71)
21 months	24/584 (4.1)	27/553 (4.9)	1.40 (0.56-3.52)
P. malariae			
9 months	7/656 (1.1)	9/627 (1.4)	0.34 (0.04-2.79)
21 months	10/584 (1.7)	5/553 (0.9)	0.04 (0.00-0.39)

The number of positives (n) of the total population examined (N). Odds ratio and 95% confidence interval based on logistic mixed models. The statistically significant results are given in bold. The *P*-values are generated from the modeled data for the combined effect of albendazole treatment over time for each of the species separately, which were significant for *P. falciparum* ($P = 0.029$) and *P. malariae* ($P = 0.016$).

Table 5. Effect of three-monthly albendazole treatment on allergy outcomes: Skin prick test to any allergens

	Placebo n/N (%)	Albendazole n/N (%)	OR (95%CI)
SPT to any allergen			
9 months	80/462 (17.3)	82/454 (18.1)	1.18 (0.74-1.86)
21 months	145/455 (31.9)	161/439 (36.7)	1.37 (0.93-2.01)

The number of positives (n) of the total population examined (N). *Odds ratio and 95% confidence interval are based on mixed effects logistic regression models. OR's and 95% CI are shown for the separate time points on SPT to any allergen. The *P*-value is generated from the modeled data for the effect of albendazole treatment overtime and no significant effects were found ($P > 0.05$).

No significant change in BMI was observed in children or in adults (Table S4). Moreover, there was no adverse effect of treatment reported.

DISCUSSION

This household-based clustered-randomized, double-blind, placebo-controlled trial shows that administering a total of seven single doses of albendazole, at three-monthly intervals, to a population living in an area of Indonesia where STH are highly prevalent, leads to decreased prevalence of helminth infections which although statistically significant, can

Table 6. Effect of three-monthly albendazole treatment on allergy outcome: Skin prick test and specific IgE to aeroallergens.

Skin prick test reactivity*	Placebo n/N (%)	Albendazole n/N (%)	OR (95% CI)
House dust mite			
9 months	36/462 (7.8)	35/454 (7.7)	1.31 (0.52-3.27)
21 months	77/455 (16.9)	76/439 (17.3)	1.37 (0.62-3.02)
Cockroach			
9 months	60/462 (13.0)	65/454 (14.3)	1.27 (0.75-2.15)
21 months	112/455 (24.6)	139/439 (31.7)	1.63 (1.07-2.50)
Specific IgE**	**N (Median, IQR)**	**N (Median, IQR)**	**β (95% CI)**
House dust mite			
9 months	391 (0.46, 0.16-2.35)	381 (0.46, 0.14-1.98)	1.01 (0.91-1.12)
21 months	339 (0.82, 0.27-3.29)	334 (0.65, 0.20-2.69)	0.93 (0.81-1.06)
Cockroach			
9 months	391 (1.47, 0.30-5.01)	381 (1.55, 0.44-4.40)	1.04 (0.93-1.16)
21 months	339 (1.83, 0.47-5.44)	334 (1.64, 0.42-4.82)	0.98 (0.85-1.14)

The number of positives (n) of the total population examined (N). *Odds ratio and 95% confidence interval based on logistic mixed models; **β (beta) and 95% confidence interval based on generalized linear mixed models from the log-transformed IgE. The values shown are back-transformed. The *P*-values are generated from the modeled data for the effect of albendazole treatment overtime and no significant effects were found ($P > 0.05$).

be taken as an incomplete reduction. The results show a transient increase in malarial parasitemia in the albendazole- compared with the placebo-treated arm in the first six months after initiation of treatment. Albendazole treatment had no statistically significant effect on the designated co-primary outcome, skin prick test reactivity to allergens.

The clinical data collected of fever and additional malaria-like symptoms, were not affected by the deworming. Clinical signs of asthma and atopic dermatitis were also not affected by albendazole treatment.

The prevalence of infection was high (>60%), which reflects the situation in many areas that are being targeted by the global deworming programs. Using a three-monthly treatment regimen which represents an extreme scenario for helminth control strategy, percentage of subjects positive for STH was reduced by 39% compared to placebo. It should be noted that in our study the sensitive PCR method has been used. The reduction in the proportion of subjects infected with hookworm and *Ascaris* was more pronounced than for *Trichuris* infections, confirming the findings using a single dose of albendazole[24]. Subjects who provided stool samples at 21 months were slightly but significantly older than those who did not. Given that hookworm infection is more prevalent in older subjects, this may have contributed to the poor deworming achieved by albendazole. The reduction achieved in worm loads, did not have any beneficial effect on BMI. Observational studies have reported that helminth infections affect growth; however randomized trials have not been consistent[25,26]. In this

regard, our study would support the outcome of a recent Cochrane review of no beneficial effect of deworming programs on nutritional indicators[27] even though it can be argued that in our study the suboptimal reduction in the STH would not allow any beneficial effect of anthelmintic in terms of BMI to be seen in the community. Importantly, the fact that the effect of such an intensive deworming strategy in a community is incomplete, needs to be considered in the agenda for the control and elimination of helminth diseases of humans[28].

Most studies on the effect of helminth infections on malarial parasitemia and clinical malaria episodes have used cross-sectional designs and have been inconclusive[6]. Longitudinal studies of anthelmintic treatment have also reported conflicting results[29,30]. A small study conducted in Madagascar has reported an increase in malarial parasitemia in levimasole treated subjects, older than 5 years of age[29], while in Nigeria, albendazole treatment of pre school children was associated with lower *P. falciparum* infection and anemia, however, the lost to follow up in this study was very high[30]. The question whether albendazole treatment during pregnancy could affect health outcomes in the offspring, was addressed in a recent report from Uganda[31]. It was found that the incidence of malaria up to one year of age was not different in the offspring of mothers born to those treated with albendazole or placebo. Our study reports the results of a community wide randomized-controlled trial that used three-monthly malarial parasitemia data obtained by microscopy. A significantly higher percentage of subjects positive for malarial parasites in the albendazole compared to the placebo arm was seen but this seemed to be a transient effect and restricted to individuals older than 15 years of age, an age group that is not the main target of the current deworming programs. The question arises as to why this effect was only seen in those >15 years of age. This could be due to the fact that *Ascaris* infection is lower in older age and therefore more easily cleared. It has been suggested that *Ascaris* is the species of helminth that has the most effect on malarial parasitemia and diseases[6]. Therefore by removing *Ascaris* in older age, we might be seeing a more profound effect on malarial parasitemia.

Using PCR, which enables detection of sub-microscopic infections at species level, it was also concluded that albendazole did not affect overall malarial parasitemia. When malaria species were analyzed separately, the percentage of subjects infected with *P. falciparum* but not with *P. vivax* increased significantly in the first 9 months post-treatment in the albendazole-treated arm, which is contrary to our hypothesis that anthelminthic treatment would reduce prevalence of malarial parasitemia[32]. It was expected that by decreasing STH, the immune hyporesposiveness would be reversed and this would be associated with stronger immune effector responses to malaria parasites. One of the possible explanations for the enhanced malarial parasitemia would be that with a reduction in STH, there is increased nutrient availability for other co infections and their growth.

It has been suggested that there are different malaria outcomes with different species of helminths; *Ascaris* being associated with protection regarding parasitemia and severity of malaria while hookworm with higher incidence of malaria[6]. Our study was not powered to conduct a stratified analysis, and with the overall gradual decrease in malaria in the study area during our study, the numbers of subjects positive for malaria parasites are too few for an ad-hoc analysis.

The findings concerning allergy outcomes, although not significant, are in line with our hypothesis that anthelminthic treatment would increase SPT reactivity. The risk of SPT reactivity increased incrementally with longer treatment and raises the question whether even longer deworming periods are needed for more pronounced effects on allergic outcomes. In support of this, a recent study reported that 15-17 years of ivermectin treatment for onchocerciasis control in Ecuador led to a significant increase in SPT reactivity to allergens[12]. In the same country, one year of anthelmintic treatment in schoolchildren did not lead to any change in SPT[10]. The question whether different species of helminths might affect allergic outcomes to a different degree, remains unanswered. It is interesting that one year anthelmintic treatment in Vietnam where hookworm infection was the prominent species, as in our study, resulted in a significant increase in SPT positivity in schoolchildren. This is in contrast to what was seen in Ecuador where *A. lumbricoides* was the most prevalent species. One common feature of the anthelmintic trials seems to be that clinical symptoms of allergy do not change with deworming. However, an important trial in pregnant women in Uganda has shown an increased risk of infantile eczema in infants of mothers treated with anthelminthics compared to those that received placebo[33]. This could indicate that exposure to worms in early life, might affect allergic outcomes more profoundly than when helminths are removed later in life[34].

One of the limitations of this trial is the overall decrease in malarial parasitemia during the two-year study period, most probably caused by actively referring subjects with malaria-like symptoms to puskesmas. Therefore further studies in areas highly endemic for malaria are needed. Treatment in the trial did result in a significant reduction in percentage of subjects infected with STH, but this reduction was incomplete. It is therefore possible that the community was insufficiently dewormed. However, our primary aim was to measure the possible effect of deworming programmes on malaria or allergy. We conclude that despite transient increase in malarial parasitemia as a result of albendazole treatment, there were no clinically relevant changes to outcome measures 21 months after treatment was initiated.

In conclusion, an extremely intensive anthelminthic treatment in a community where STH are highly endemic, does not lead to elimination but reduces both prevalence and intensity of helminths. Such MDA regiment appears safe and does not lead to any significant change with respect to malaria infections or allergies. However, it is worrying that such vigorous community treatment does not have a more pronounced effect on STH burden. Better integrated control strategies would be needed to deworm and subsequently assess whether the risk for malaria infections or allergies change.

REFERENCES

1 Bethony J, Brooker S, et al. Soiltransmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521–32.

2 Utzinger J. A research and development agenda for the control and elimination of human helminthiases. PLoS Negl Trop Dis 2012;6:e1646.

3 Lustigman S, Prichard RK, et al. (2012) A research agenda for helminth diseases of humans: the problem of helminthiases. PLoS Negl Trop Dis 2012;6:e1582.

4 Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol 2011;11:375–88.

5 Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733–44.

6 Nacher M. Interactions between worms and malaria: Good worms or bad worms? Malar J 2011;10:259.

7 Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. N Engl J Med 2002;347: 911–20.

8 Feary J, Britton J, et al. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66: 569–78.

9 Flohr C, Quinnell RJ, et al. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009;39:20–32.

10 Cooper PJ, Chico ME, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006;367:1598–1603.

11 Flohr C, Tuyen LN, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2010;40:131–42.

12 Endara P, Vaca M, et al. Long-term periodic anthelmintic treatments are associated with increased allergen skin reactivity. Clin Exp Allergy 2010;40:1669–77.

13 Lau S, Matricardi PM. Worms, asthma, and the hygiene hypothesis. Lancet 2006;367:1556–8.

14 Hamid F, Wiria AE, et al. A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2011;11:83.

15 Wiria AE, Prasetyani MA, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2010;10:77.

16 Harijanto PN. Malaria treatment by using artemisinin in Indonesia. Acta Med Indones 2010;42:51–6.

17 Elyazar IR, Hay SI, et al. Malaria distribution, prevalence, drug resistance and control in Indonesia. Adv Parasitol 2011;74:41–175.

18 Szefler SJ. Advances in pediatric asthma in 2007. J Allergy Clin Immunol 2008;121:614–9.

19 World Health Organization. Guidelines for the treatment of malaria, 2nd ed. World Health Organization 2010.

20 Adegnika AA, Verweij JJ, et al. Microscopic and sub-microscopic Plasmodium falciparum infection, but not inflammation caused by infection, is associated with low birth weight. Am J Trop Med Hyg 2006;75:798–803.

21 WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-forheight and body mass index-for-age: Methods and development. Geneva:World Health Organization 2006;312.

22 Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963–74.

23 R-forge website. Available: http://lme4.r-forge.r-project.org/

24 Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. JAMA 2008;299:1937–48.

25 Alderman H, Konde-Lule J, et al. Effect on weight gain of routinely giving albendazole to preschool children during child health days in Uganda: cluster randomised controlled trial. BMJ 2006;333:122.

26 Dickson R, Awasthi S, et al. Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. BMJ 2000;320: 1697–701.

27 Taylor-Robinson DC, Maayan N, et al. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators,

haemoglobin and school performance. Cochrane Database Syst Rev 2012;7:CD000371.

28 Prichard RK, Basanez MG, et al. A research agenda for helminth diseases of humans: intervention for control and elimination. PLoS Negl Trop Dis 2012;6:e1549.

29 Brutus L, Watier L, et al. Confirmation of the protective effect of Ascaris lumbricoides on Plasmodium falciparum infection: results of a randomized trial in Madagascar. Am J Trop Med Hyg 2007;77:1091-5.

30 Kirwan P, Jackson AL, et al. Impact of repeated four-monthly anthelmintic treatment on Plasmodium infection in preschool children: a double-blind placebo-controlled randomized trial. BMC Infect Dis 2010;10:277.

31 Webb EL, Mawa PA, et al. Effect of single-dose anthelmintic treatment during pregnancy on an infant's response to immunisation and on susceptibility to infectious diseases in infancy: a randomised, double-blind, placebo-controlled trial. Lancet 2011;377: 52–62.

32 Specht S, Hoerauf A. Does helminth elimination promote or prevent malaria? Lancet 2007;369:446–7.

33 Mpairwe H, Webb EL, et al. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 2011;22:305-12.

34 Djuardi Y, Wammes LJ, et al. Immunological footprint: the development of a child's immune system in environments rich in microorganisms and parasites. Parasitology 2011;138:1508–18.

SUPPLEMENTARY METHODS

Additional information on the study area and procedures

Ende district, an area highly endemic for STH, is situated near the equator (8°45'S, 121°40'E) and it is characterized by a uniform high temperature, in the range of 23-33.5 °C, with humidity of 86-95%. Average yearly rain fall is 1.822 mm with about 82 rainy days, especially from November to April, with the peak in December until March. The semi-urban village of Nangapanda, endemic for malaria, had a population of 3583 and is located in the coastal area with most villagers being farmers and fishermen with some government officers or private sector employees. The rural village Anaranda had 1631 inhabitants and is located 80 km further inland of Nangapanda. There was poor infrastructure and inhabitants generated income mainly from farming.

Regarding the availability of the anthelminthics in the community, there was no deworming campaign in this area during the study period. Pyrantel pamoat (Combantrin®) and dehydropiperazine (Bintang 7 puyer 17®) were the only available anthelminthics in the market. The local primary health centre (Puskesmas) did not provide the current trial study participants by any anthelminthic treatment but referred them to the trial team.

Malaria control, such as by artemisinin-combination therapy (ACT) treatment and insecticide-treated nets (ITN) or long-lasting insecticide-treated nets (LLIN) although planned, were not implemented yet during our study period. This was due to several difficulties faced in some parts of Indonesia, such as instable drug supply, lack of training on definitive diagnosis of malaria by the laboratory staff, as well as insufficient bednet supply and poor compliance[17]. Malaria drugs such as chloroquine and quinine were available in the shops, however, little information is available on proper self medication. Therefore, before and during the study period, regular training of field workers was undertaken on how to prevent malaria (use of repellent and bednet, irrigation of breeding places) and how to treat malaria (not to self medicate but to visit puskesmas for diagnosis and treatment). Indoor residual spraying was done by the local health authority for dengue control against an outbreak at the beginning of 2008.

The treatment of suspected malaria cases at the puskesmas was chloroquine and primaquine for *P. vivax*, while for *P. falciparum* sulfadoxine/pyrimethamine was commonly used. Subjects in our study with fever and/or any one of the malaria-like symptoms (see below for detailed description) were referred to the puskesmas for assessment and treatment according to local health center policy.

The anthelmintic treatment and placebo were coded and the code was concealed from trial investigators and participants. The tablets were distributed by trained health workers and the intake was directly observed. Labels with the study subject ID were printed from a computer database and attached to the appropriate strip of treatment by a separate team located in Jakarta without the involvement of the study investigators. In order to assess whether anthelminthic treatment had any adverse effect on the growth of children or on the incidence of allergy, interim analyses were done at one year post-treatment by a

monitoring committee. After completion of the study the whole population was treated with albendazole (a single dose of 400mg for three consecutive days).

The malaria slides were read by microscopy at the Department of Parasitology in Jakarta. The quality control for microscopic reading took place in the pilot phase of the project. In cooperation with NAMRU-2 (US Naval Medical Research Unit-2) two microscopists from our team were trained, inter-observer differences were assessed and following satisfactory training they were certified. At the pilot phase, and throughout the study, PCR was used to monitor the microscopy data with a high degree of agreement between microscopy and PCR. In a random sub-sample at 9 months and 21 months post-treatment we measured malarial parasitemia by PCR.

Primers and the *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*-specific probes were used with some modifications in the fluorophore- and quencher-chemistry. Amplification reactions of each DNA sample are performed in white PCR plates, in a volume of 25 µl with PCR buffer (HotstarTaq master mix), 5 mmol/l MgCl2, 12.5 pmol of each Plasmodium-specific primer and 15 pmol of each PhHV-1-specific primer, 1.5 pmol of each *P. falciparum*, *P. vivax*, *P. malariae*-specific XS- probes, and PhHV-1-specific Cy5 double-labelled detection probe, and 2.5 pmol of each *P. ovale*-specific XS-probes (Biolegio), and 5 µl of the DNA sample were used. Amplification consists of 15 min at 95°C followed by 50 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Amplification, detection, and analysis are performed with the CFX96 real-time detection system (Bio-Rad laboratories). The PCR output from this system consists of a cycle-threshold (Ct) value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, and reflecting the parasite-specific DNA load in the sample tested. Negative and positive control samples are included in each amplification run.

Stool samples were collected and preserved in 4% formaldehyde for microscopy examination or frozen (-20°C) unpreserved for PCR detection. The formol-ether acetate concentration method was performed on the formalin preserved stool samples followed by microscopic examination for Trichuris trichiura infections. As described in detail before[15], DNA was isolated from approximately 100 mg unpreserved feces and examined for the presence of *Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides and Strongyloides stercoralis* DNA by the multiplex qPCR. The qPCR output from this system consisted of a Ct value; negative and positive control samples were included in each run of the amplification. Positive Ct- values were grouped into three categories: Ct<30.0, 30.0≤Ct<35.0 and ≥35.0 representing a high, moderate and low DNA load, respectively.

Data collection on clinical symptoms

A year before the study enrolment, community workers were recruited and trained in taking finger-prick blood for the three-monthly malarial parasitemia survey in Nangapanda, observing drug intake, recording adverse treatment effects, as well as measuring fever and administering monthly malaria-like symptoms questionnaire. These questionnaires were based on WHO definitions[19] and were assessed in all individuals that were present at the time of the survey. Subjects with fever (≥37.5°C) or additional malaria-like symptoms (headache,

fatigue and nausea) at the time of visits were referred to the puskesmas for treatment according to local standard protocols. The monthly data on fever (≥37.5ºC, using digital thermometer) and additional malaria-like symptoms were collected at baseline September 2008 and in the months Oct 08, Nov 08, Dec 08, Jan 09, Feb 90, March 09, Apr 09, May 09, June 09, Aug 09, Sept 09, Oct 09, Nov 09, Dec 09, Jan 10, Feb 10, March 10 and Apr 10. At baseline, 1396 individuals were assessed in placebo and 1381 in the albendazole arm and at the last timepoint, 1165 and 1181 subjects were followed up in the two groups, respectively. Questionnaire data were available for all timepoints from 45.8% and 47.2% of placebo and albendazole group whereas data for 80% of the timepoints were available from 83.8% and 87.6% of the two groups, respectively. The number of events was recorded in total of 15259 and 15307 person months at risk for placebo and albendazole groups, respectively.

The modified video-assisted (for asthma symptoms) and illustration-assisted (for atopic dermatitis) ISAAC questionnaire, translated to Bahasa Indonesia and back translated for use in our studies within the EU funded project GLOFAL (www.glofal.org), were administered at baseline and at 21 month timepoints. Data were available from 629 in placebo and 635 in albendazole arm at baseline, while these numbers were 460 and 445, respectively, at the 21 month timepoint. These questionnaires were administered to the parents/guardians of subjects who were skin prick tested with allergens: the trial profile is given in supplementary Figure 1B. The prevalence of asthma symptoms were obtained from the following questions: (i) has your child ever had asthma? (ii) has your child ever been diagnosed for asthma by a doctor? and (iii) has your child in the past 12 months had wheezing or whistling in the chest?; while the prevalence of atopic dermatitis was obtained from the questions: (i) has your child ever had doctor/paramedic diagnosed allergic eczema and (ii) has your child ever had one or more skin problems accompanied by an itchy rash?

If the answer to one or more of these questions was positive, the subjects were considered to have either asthma or atopic dermatitis symptoms.

Detailed description of the statistical models used

Descriptives were computed for each variable (mean and standard deviation or median and interquartile range for continuous outcomes, numbers and percentages for categorical variables). For children ≤ 19 years, BMI age-standardized z-scores were calculated according to WHO references[21].

Two sources of correlation among observations should be accounted for when modeling these data, namely observations at various timepoints for a subject are correlated due to subject specific effects and observations within households are correlated due to environmental effects shared within households. To model these correlations we used random effects. For subject effects a random intercept and a random slope were used, i.e. each subject has its own intercept and slope, where the latter models the change of the outcome variable over time. Observations within a household also have a shared random intercept. Thus the intercept for an observation of a specific subject from a specific household is the overall mean plus the

subject specific effect plus the household effect. By doing so correlation among observations of the same household was modeled since these observations share the same household effect. To assess treatment effects generalized linear mixed models[22] were used where the term "mixed" corresponds to the used random effects. Unless stated otherwise all models included area as covariate in the model to take into account the differences between the two villages. Generalized linear mixed models provide a flexible and powerful tool to derive valid inference while capturing the data correlations induced by clustering within households and repeated evaluations in time of the same subject.

For continuous outcome variables which were measured at 0, 9 and 21 months, treatment effects were modeled at timepoint 9 and 21 months, because treatment started at 0 months and the design is a randomized trial no treatment effect should be present at time 0. We allowed for different treatment effects at 9 and 21 months. Beta's and 95% confidence intervals are provided for 9 and 21 months. The betas represent the mean difference between the placebo and treatment group. An overall test for treatment effect over time was performed by using a likelihood ratio test which compares the model with and without the treatment effect (2 df test).

For binary outcome variables measured at 9 and 21 months, the logit link was used (mixed effect logistic regression). In these models only the two random intercepts were included and the random subject specific slope was omitted. Odds ratios and 95% confidence intervals are reported. Analogously to continuous outcome variables two degrees of freedom likelihood ratio tests were performed to assess treatment effects over time. Note that the model based odds ratios are different from crude odds ratios directly computed from the sample due to missing observations and due to the presence of random effects and the covariate area in the model. Malarial parasitemia by microscopy was measured at a three monthly basis. To model these data, similar models were used. Specifically at each of the seven timepoints (excluding time zero) a treatment effect was included. The likelihood ratio test for treatment effect over time has therefore 7 degrees of freedom. All generalized linear mixed models were fitted using the lme4 package (Douglas Bates, Martin Maechler and Ben (2011). lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4) in R[23].

For each malaria-like symptom (fever, headache, fatigue, and nausea), total number of events and person months were computed for each treatment group. We calculated incidence rates for all events. Symptom episodes within three months of an initial presentation with the same symptom were regarded as part of the same episode. Hazard ratios for effect of treatment were calculated with Cox regression with robust SE to allow for within-subject and within household clustering (STATA 12).

SUPPLEMENTARY TABLES

Table S1. Effect of three-monthly albendazole treatment on helminth infection

	Placebo n/N (%)	Albendazole n/N (%)	OR (95% CI)
Helminth infection (any spp)			
9 months	395/477 (82.8)	247/480 (51.4)	0·07 (0·04-0·11)
21 months	353/448 (78.8)	172/411 (41.9)	0·05 (0·03-0·08)
Hookworm[1]			
9 months	359/524 (68.5)	161/508 (31.7)	0·02 (0·01-0·04)
21 months	305/466 (65.5)	99/423 (23.4)	0·01 (0·01-0·03)
A. lumbricoides[1]			
9 months	174/524 (33.2)	65/508 (12.8)	0·24 (0·16-0·36)
21 months	140/466 (30.0)	41/423 (9.7)	0·18 (0·11-0·29)
T. trichiura[2]			
9 months	219/726 (30.2)	160/673 (23.8)	0·58 (0·42-0·80)
21 months	177/633 (28.0)	101/571 (17.7)	0·40 (0·28-0·58)

The number of positives (n) of the total population examined (N).[1]diagnosed by PCR. [2]diagnosed by microscopy. Odds ratio and 95% confidence interval based on logistic mixed models. The *P*-values are generated from the modeled data for the combined effect of albendazole treatment over time, which were significant ($P < 0.001$) for any helminth and for each of the species separately.

Table S2. The effect of albendazole on fever and additional malaria like symptoms

	Placebo		Albendazole			
	Events (PM)	Incidence per PM	Events (PM)	Incidence per PM	Unadjusted IRR	Adjusted IRR
Fever	414 (18494)	0.02	429 (18636)	0.02	1.03	1.03
Headache	333 (19067)	0.02	340 (19563)	0.02	1	1
Fatigue	49 (22362)	0.002	69 (22535)	0.003	1.39	1.41
Nausea	76 (21749)	0.003	55 (22211)	0.002	0.71	0.71
Any symptom	661 (15259)	0.04	690 (15307)	0.05	1.04	1.04

IRR: incidence rate ratio. PM: Person months. Adjusted with age and sex. The *P*-values are generated from Cox regression of albendazole treatment over time with robust SEs to allow for within-subject and within household clustering and no significant effects were found ($P > 0.05$).

Table S3. Reported clinical symptoms of allergy

	Placebo n/N (%)	Albendazole n/N (%)	OR (95% CI)
Asthma			
21 months	8/461 (1.7)	11/445 (2.5)	1.11 (0.07-17.26)
Atopic dermatitis			
21 months	13/461 (2.8)	9/445 (2.0)	0.57 (0.16-2.02)

The number of positives (n) of the total population examined (N). The *P*-values are generated from the modeled data for the effect of albendazole treatment after 21 months and no significant effects were found ($P > 0.05$). At baseline 8/692 (1.2%) and 18/692 (2.6%) in the placebo group reported symptoms of asthma and atopic dermatitis, respectively, while in Albendazole this was 10/635 (1.6%) and 11/635 (1.7%).

Table S4. Effect of three-monthly albendazole treatment on BMI

	Placebo N (Median, IQR)	Albendazole N (Median, IQR)	β (95% CI)
BMI			
9 months	498 (22.42, 19.91 - 25.54)	499 (22.07, 19.96 - 24.56)	-0·10 (-0·29-0·09)
21 months	430 (22.42, 19.68 - 25.56)	425 (21.56, 19.44 - 24.12)	-0·15 (-0·39-0·10)
z-BMI			
9 months	346 (-0.81, -1.44 - -0.13)	334 (-0.96, -1.56 - -0.30)	-0·04 (-0·17-0·09)
21 months	272 (-1.29, -2.21 - -0.56)	269 (-1.57, -2.32 - -0.74)	-0·07 (-0·23-0·10)

The total population examined (N). IQR = Interquartile range. β (beta) and 95% confidence interval based on generalized linear mixed models. The *P*-values are generated from the modeled data for the combined effect of albendazole treatment over time and no significant effects were found ($P > 0.05$). Baseline data are shown in Table 1 of the manuscript.

SUPPLEMENTARY FIGURES

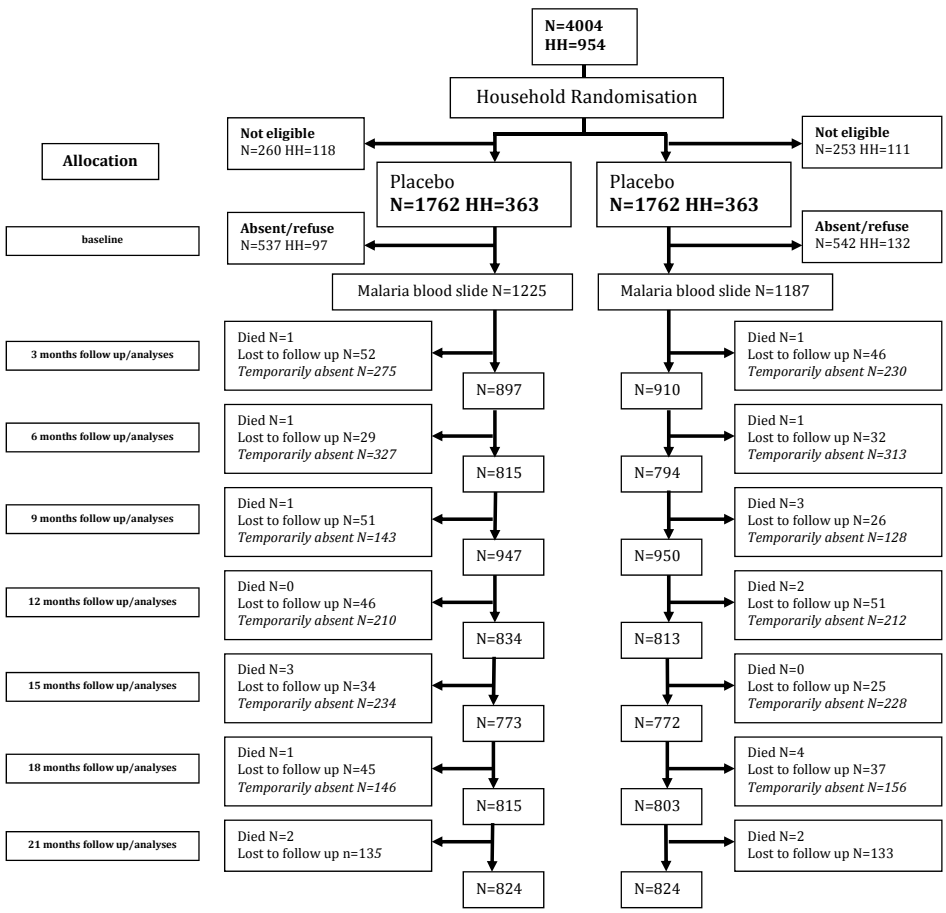


Figure S1A. Profile of trial with malarial parasitemia as outcome in the village of Nangapanda where malaria is endemic

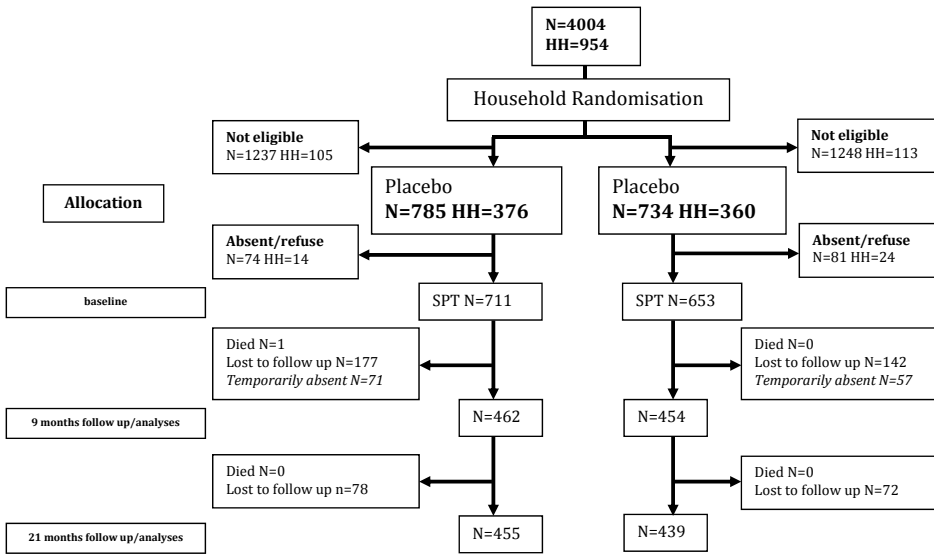


Figure S1B. Profile of trial with skin prick test (SPT) reactivity as outcome in children 5-15 years of age in both Nangapanda and Anaranda

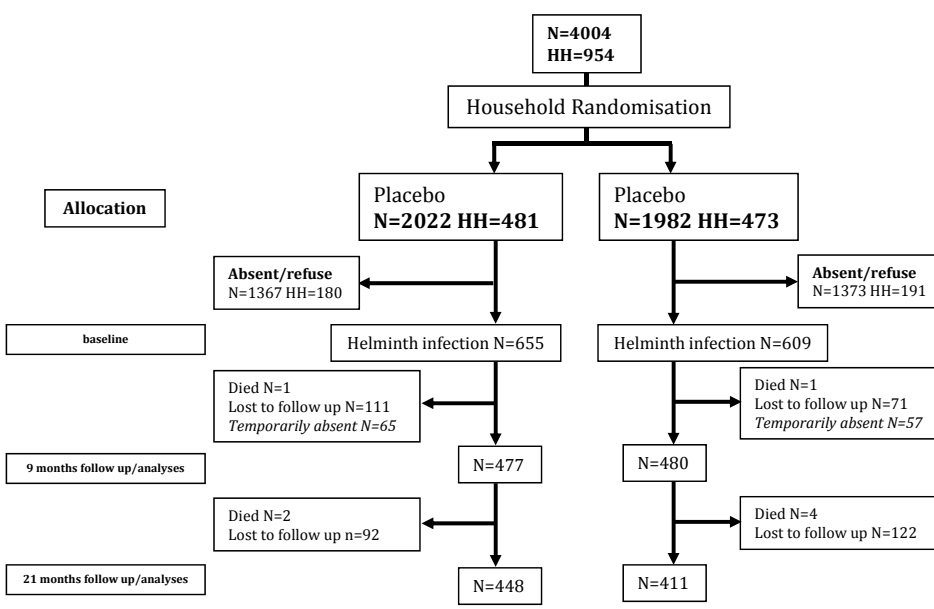


Figure S1C. Profile of trial with helminth infection as outcome in villages of Nangapanda and Anaranda

6

MOLECULAR DIAGNOSTICS IN A HELMINTH-ENDEMIC AREA IN INDONESIA PROVIDES LEADS FOR THE LACK OF CLINICAL ALLERGY

Firdaus Hamid[1,2], Serge A Versteeg[3], Aprilianto E Wiria[2,4], Linda J Wammes[2], Sitti Wahyuni[5], Tamiawati Supali[4], Erliyani Sartono[2], Ronald van Ree[3,6,*], Maria Yazdanbakhsh[2,*]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
4. Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia
5. Department of Parasitology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
6. Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

*these authors contributed equally

Manuscript in submission

ABSTRACT

Background Helminth infections induce Th2 immunity with strong IgE responses, against parasite antigens and common allergens. Yet chronic helminth infections have been reported to be inversely associated with skin reactivity and clinical allergy.

Aim Molecular characterization of IgE responses against common allergen sources in a helminth-endemic area.

Methods A selection of 150 sera of school children from Flores Island, Indonesia, previously tested by ImmunoCAP and skin prick test (SPT) for IgE against house dust mite (HDM), cockroach, grass pollen (no SPT), peanut and shrimp were tested for IgE against 112 purified natural allergens by ImmunoCAP-ISAC microarray. ImmunoCAP inhibition was used to assess the role of cross-reactive carbohydrate determinants (CCD).

Results Of the 150 sera, 65% to 90% were positive by ImmunoCAP for the common inhalant and food allergen sources. At the molecular level, IgE against established major allergens was <10% for HDM and cockroach and absent for peanut and shrimp. Only for grass pollen (and tree pollen and walnut), IgE was detected in approximately half of the children, but selectively against natural glycoproteins. ImmunoCAP inhibition showed that CCD is at the basis of these IgE responses, most likely induced by helminths. Only venom allergens were frequently recognized without carrying CCD.

Conclusions ImmunoCAP-ISAC has revealed that in helminth-endemic areas, CCD contributes significantly to high sensitization levels to common allergen sources. Purified natural plant glycoproteins consequently have a negative impact on the specificity of such microarrays. These cross-reactive IgE responses are most likely part of the explanation of the inverse association between helminth infections and clinical allergy.

INTRODUCTION

The prevalence of asthma and allergic diseases has increased in affluent countries over recent decades, and the same development has now begun in many non-affluent countries[1]. At the same time however, helminth infections remain one of the major health problems in these countries, especially in tropical regions, where such infections are linked to poverty and rural living[2]. Both helminths and allergens are potent inducers of T helper 2 (Th2) responses that lead to high levels of immunoglobulin (Ig) E, mast cells, tissue eosinophilia and the secretion of Th2 cytokines such as interleukin (IL)-4, IL-5, IL-9 and IL-13[3].

Despite the similar immunological profiles of helminth infections and allergy, several cross-sectional studies have suggested a protective effect of helminth infection on atopic disorders[4,5], with a number of intervention studies supporting this notion[6-9]. It has been reported that high intensity helminth infection protects subjects from skin test reactivity to allergens[10,11]. Although not all studies confirm this, it has been proposed that the negative association between helminth infections and allergies might be the result of the strong immune-regulatory cell networks induced by chronic helminth infections that prevent pro-inflammatory Th2 responses from triggering disease in target organs[12,13]. However, there is also evidence for a parallel mechanism: the possibility that helminth infections are accompanied by IgE responses that are cross-reactive between helminth antigens and common environmental and/or food allergens, but of low biological activity and therefore associated with low or negligible skin prick test (SPT) reactivity to such cross-reactive allergens[14-16].

Laboratory diagnostics and skin test reagents to support the diagnosis of allergy have until recently always been based on allergen extracts. The increasing knowledge on the serological and clinical role of individual allergenic molecules in these extracts and their availability as purified natural or recombinant reagents has opened new diagnostic avenues. The introduction of so-called component-resolved diagnosis (CRD) based on individual allergen molecules has been instrumental in improving the clinical prognostic relevance of diagnostic tests and in identifying the origin of sensitization. Some individual allergen molecules have proven to be better predictors of clinical allergy[17] than whole extracts and have been shown to be risk factors for severe clinical phenotypes[18]. In addition, we now know that some allergens are biomarkers for primary sensitization to particular types of allergen sources, like group 5 allergen (e.g. Phl p 5) for grass pollen, group 1 and 2 allergens (e.g. Der p 1 and Der p 2) for house dust mite, and Ara h 2 for peanut sensitization[19]. On the other hand, cross-reactive structures have been identified that are present in many inhalant and food allergens sources, leading to positive IgE outcomes for many extract-based tests, without really revealing whether recognition is based on primary sensitization and/or cross-reactivity. The most relevant broadly cross-reactive structures are the pathogenesis-related (PR)-10 proteins (i.e. Bet v 1 and homologues in fruits and tree nuts)[20], profilins[21], tropomyosins[22] and lipid transfer proteins[23]. A special cross-reactive structure is not of protein but of carbohydrate nature, i.e. cross-reactive carbohydrate determinants (CCD)[14,24]. IgE against CCD has a very broad spectrum of cross-reactivity across the plant kingdom but also to glyco-proteins of invertebrate animals such as insects (venoms)

and parasites. Sensitization to CCD has first been shown to find its origin in pollen, bee and wasp venom allergies. In hay fever patients with strong pollen sensitization, IgE against CCD is most common and often accompanied by sensitization to profilin. It has been demonstrated convincingly that CCD-specific IgE is of poor biological activity and consequently of no clinical relevance[25]. Later it was suggested that helminth infections in humans and in cattle can play an important role in sensitization to CCD[16,26].

Many biomarkers for primary sensitization as well as cross-reactive allergens are now available as single diagnostic reagents on e.g. ImmunoCAP, but more recently, protein microarray technology has entered the field of molecular allergology, i.e. the ImmunoCAP-ISAC microarray with 112 individual purified natural and recombinant inhalant and food allergens[27,28]. This technology allows testing for individual IgE recognition profiles with a limited volume of serum, providing comprehensive information about likely sources of primary sensitization and cross-reactivity. For epidemiological research such technology has proven to provide an enormous resource of data[29] that can help elucidate the process of sensitizations and identify risk and protective factors for clinical phenotypes[30,31].

To the best of our knowledge, the present study is the first to characterize IgE profiles to allergen components using the ImmunoCAP-ISAC technology in a developing country in an area where helminths are endemic. Our aim was to elucidate the molecular basis of the extremely high prevalence of sensitization to common allergen sources (65% - 85%) but low SPT reactivity and virtually absent clinical allergy, observed amongst Indonesian school children.

METHODS

Study population

This study was part of a longitudinal study investigating the effect of anthelminthic treatment on malarial parasitemia and allergy, described in detail elsewhere[32,33]. In short, the study was conducted between 2008 and 2010 in a semi-urban (Nangapanda) and a rural (Anaranda) site, both located on Flores Island, where the infrastructure, access to electricity, shops and health care were low in the latter. A total of 1674 schoolchildren aged 5-15 years old were included in the study of allergic outcomes. Children were asked to undergo skin prick testing, provide blood for serum IgE testing, and a stool sample for diagnosis of soil-transmitted helminth infections. In addition, parents or guardians were asked to fill out a questionnaire to record self-reported allergic symptoms. Written informed consent was obtained from a parent or guardian of each child. This investigation was carried out within the ImmunoSPIN programme (www.immunospin.org) and approved by the Ethical Committee of the Medical Faculty, University of Indonesia, Jakarta and has been filed by the ethics committee of the Leiden University Medical Center, the Netherlands. The study was registered as clinical trial (ISRCTN83830814).

Due to budgetary restrictions, 150 serum samples could be analyzed in the current study. These were selected, based on plasma availability and complete information regarding skin prick test (SPT) reactivity, clinical symptoms of allergy as well as ImmunoCAP IgE data.

Questionnaires

Symptoms of asthma and atopic dermatitis in the past 12 months were recorded using a modified version of the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire as reported before[33].

Skin prick test

House dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*), peanut, shrimp (kindly provided by Paul van Rijn from HAL Allergy Laboratories, Leiden, The Netherlands) and cockroach (*Blattella germanica*; Lofarma, Milan, Italy) were used for SPT. The SPT was considered positive when a wheal size was more 3 mm as described[33].

IgE antibody measurement

ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) was used to measure IgE against house dust mite (*D. pteronyssinus*), cockroach (*B. germanica*), grass pollen (*Phleum pretense*), bromelain (*Ananas comosus*), peanut and shrimp, following to the manufacturer's instructions. Levels of ≥ 0.35 kU_A/L were considered positive.

For multiplex analysis, the ImmunoCAP-ISAC 112 with 112 allergen molecules (ThermoFisher Scientific) was used following manufacturer's instructions. Briefly, arrays were incubated with 30 µL of plasma for 2 hours. After washing and drying, arrays were incubated for 30 minutes with fluorescently labeled anti-human IgE. After further washing, arrays were read with a laser scanner. Results were expressed as ISAC standardized units (ISU)/L. Levels of >0.3 ISU/L were considered as positive.

Total IgE was measured by ELISA as described previously[32,33]. The results were expressed in International Units (IU/ml).

IgE inhibition assay

For ImmunoCAP inhibition assays, 4 separate pools of plasma were prepared, a HDM-, a cockroach-, a grass pollen- and a peanut-positive pool which each contained equal volumes (n=16, n=11, n=3, and n=18, respectively) of plasma samples that were positive (IgE levels ≥5 kU_A/L) for the respective allergen sources. Inhibitors used were bromelain (marker for CCD), SEA (soluble egg antigen of *Schistosoma haematobium*, a source of helminth CCD)[16] and *Ascaris lumbricoides* antigen (locally endemic parasite). Each 75 µL of pooled plasma was mixed with an equal volume of inhibitor dilutions (titrated inhibition) and pre-incubated at room temperature for 1 hour. Subsequently, samples were analyzed for HDM-, cockroach-, grass pollen- and peanut-specific IgE, following the normal ImmunoCAP protocol. Results were expressed as percentages of an uninhibited control (PBS)[16].

Parasitological examinations

Trichuris trichiura was detected by microscopy and a multiplex real-time PCR was used for detection of hookworms (*Ancylostoma duodenale*, *Necator americanus*), *A. lumbricoides*, and *Strongyloides stercoralis* DNA as detailed previously[32,33].

Statistical analyses

Statistical analyses were performed using IBM Statistical Package for Social Sciences (IBM Corp., Armonk, New York, USA) version 20. Descriptive data were expressed as means (± standard deviations), frequency (percentage of measured data) and geometric means [95% confidence intervals (CI)]. The correlation of IgE levels between natural or recombinant allergens or whole allergen extracts and CCD was examined with Spearman rank correlation coefficients. Logistic regression was used to examine the association between helminth infection and IgE reactivity to allergen component measured on ImmunoCAP-ISAC as well as the association between helminth infection and IgE reactivity to whole allergen extract tested on ImmunoCAP with adjustment for age, sex, and area. A *P-value* < 0.05 was considered statistically significant.

RESULTS

Sensitization by extract ImmunoCAP and SPT

Of the 150 sera selected for ImmunoCAP-ISAC, sensitization to cockroach, as assessed by extract ImmunoCAP, was highest (86.7%), followed by shrimp (79.3), HDM (74%) and peanut (64.7%). However, skin prick reactivity was much lower and highest for HDM (26.7%), followed by cockroach (24%), peanut (5.5%) and shrimp (4.7%). Total IgE was high with a geometric mean of 2816.4 IU/ml (95% CI, 2256.5-3515.2) (Table 1).

In this group, wheeze or eczema in the past 12 months was reported for only 5 children. None of the study subjects reported any symptoms of food allergy. With such small numbers it was not possible to use these data in further statistical analyses.

In this group of 150 subjects, 74 had stool samples and the prevalence of helminth infections was 93.2% (69/74). Analyzing characteristics of subjects providing stool samples and those who did not, showed no differences in atopic sensitization (SPT and IgE), age and sex.

Sensitization by ImmunoCAP-ISAC microarray

A total of 112 (74.7%) children recognized at least one allergen component. Of 112 allergens tested by ISAC, 46 allergens were recognized (Figure 1 and Table S1). The prevalence of recognition of purified natural glycosylated allergens was highest for those from grass pollen (nPhl p 4; 53.3%; nCyn d 1: 50.7%), followed by those from walnut (nJug r 2: 32.0%), and tree pollen (plane nPla a 2: 30.7%; cypress nCup a 1: 21.3%; Japanese cedar nCry j 1: 18,7%). IgE to MUXF3 representing the CCD glycan structures commonly found on these glycoproteins was detected in 21.3%. Recombinant allergens and non-glycosylated

Table 1. Characteristic of the study population

	N	n (%)
Age (mean in years, SD[#])	150	10.6 (2.9)
Sex (female)	150	83 (55.3)
Skin prick test		
House dust mite	150	40 (26.7)
Blattella germanica (cockroach)	150	36 (24.0)
Peanut	150	8 (5.3)
Shrimp	150	7 (4.7)
Specific IgE (cut off ≥ 0.35 kU_A/L)		
Dermatophagoides pteronyssinus	150	111 (74.0)
B. germanica	150	130 (86.7)
Peanut	150	97 (64.7)
Shrimp	150	119 (79.3)
Phleum pretense (grass pollen)	30	27 (90)
Ananas comosus (bromelain)	30	26 (86.7)
Total IgE (geometric mean, 95% CI)	133	2816.4 (2256.5-3515.2)
Helminth infection (n, %)		
Any helminths	74	69 (93.2)
Hookworms[1]	76	58 (76.3)
Ascaris lumbricoides[1]	76	32 (42.1)
Trichuris trichiura[2]	104	30 (28.8)

[#] SD: standard deviation. CI: confidence intervals. Helminth infection was diagnosed by [1]PCR, [2]microscopy.

natural allergens (walnut nsLTP, cockroach and shrimp tropomyosin) not substituted with CCD-type glycans (n=28) was found in < 3.4% with the exception of one recombinant tree pollen allergen (rPla a 1: 12.7%) and three recombinant venom allergens (honey bee rApi g 1: 18.7%; paper wasp rPol d 5: 36.7%; common wasp: 34.0%).

Mono sensitization was found in 12 children (8%), with 3 subjects positive for recombinant plane tree (rPla a 1), 3 subjects positive for paper wasp (rPol d 5), 2 subjects positive for nCyn d 1 and the other 4 subjects recognized different type of allergens (nPhl p 4, saltwort weed pollen [nSal k 1], dog [rCan f 2] and latex [rHev b 5]).

Correlation between IgE to natural allergens and CCD

The correlation of IgE (ImmunoCAP-ISAC) to natural glycosylated allergens nJug r 2, nCyn d 1, nPhl p 4, nCup a 1, and nPla a 2 and to CCD (MUXF3) was high: r = 0.77, 0.66, 0.72, 0.60, and 0.74, respectively; $P < 0.001$ (Figure S1). IgE levels to both extracts of grass pollen and peanut detected by ImmunoCAP were highly correlated with those against bromelain IgE (r=0.87 and 0.84, respectively; $P < 0.001$, Figure S2a-b). We found a weak correlation

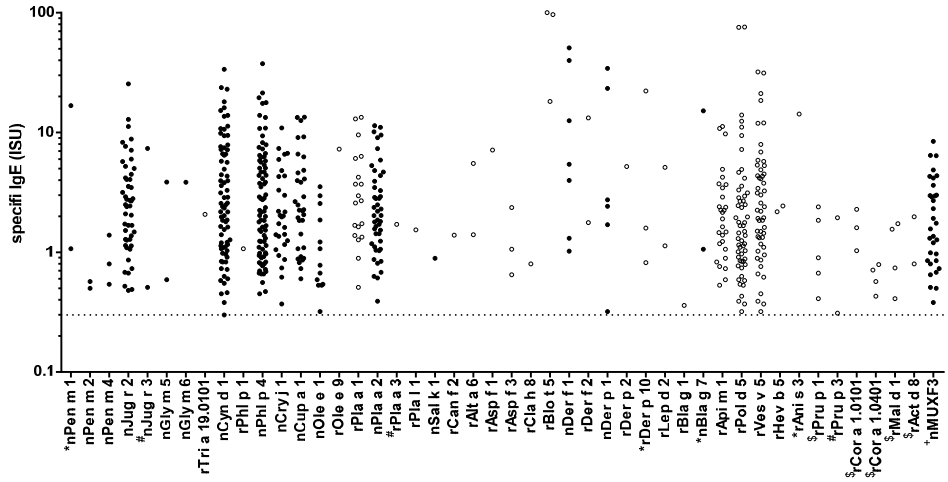


Figure 1. The levels of IgE to purified natural and recombinant allergens recognised on ImmunoCAP-ISAC (N=150). *Solid circles represent natural (n) component allergens, open circles represent recombinant (r) component allergens. Cross reacting allergen to: *tropomyosin, #non-specific lipid transfer protein (nsLTP), $the pathogenesis-related (PR)-10 protein, +Cross reactive carbohydrate determinants (CCDs). Each circle represents a single positive subject and the dotted line indicates the cut-off value for the ISAC assay (0.3 ISU/L). ISU, ISAC Standardized Unit. The allergen abbreviations are given in Table S1*

between bromelain IgE and IgE levels to cockroach (r= 0.53; P = 0.002) and a trend for HDM (r=0.32; P = 0.085) (Figure S2c-d).

IgE inhibition assay

Titrated CAP inhibition assays demonstrated that binding of IgE from peanut- and grass pollen-pooled samples was inhibited by bromelain (76.1% and 57.5% inhibition, respectively) and SEA (76.3% for peanut-pooled IgE and 45% for grass pollen-pooled IgE) whereas this was much less when the endemic helminth *A. lumbricoides* antigen was used for inhibition (25% for peanut-pooled IgE and 17% for grass pollen-pooled IgE) (Figure 2a-b). In HDM- and cockroach-pooled plasma; bromelain, SEA and *Ascaris* antigens could only inhibit up to 25% binding of IgE (Figure 2a-c).

Comparison of aero- and food-allergens measured on ImmunoCAP and ImmunoCAP-ISAC

Using ImmunoCAP, IgE sensitization to whole extract of HDM was detected in 74% of the study subjects. When the same samples were tested against HDM components on ImmunoCAP-ISAC, we found only up to 5% of the subjects showed IgE reactivity specific to natural or recombinant component of house dust mite (Figure 3a).

Of 113 subjects positive for IgE against cockroach extract on ImmunoCAP, only 2% of the children were reactive to cockroach allergen components; either nBla g 7 (1.3%) or rBla g 1 (0.7%) on ImmunoCAP-ISAC (Figure 3b). Peanut reactivity on CAP was observed

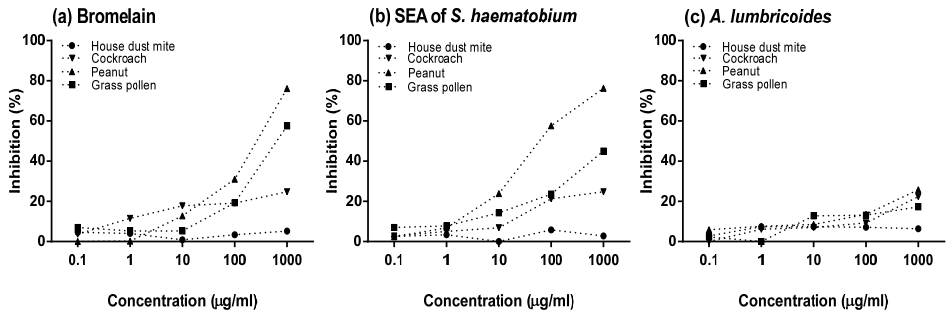


Figure 2. IgE inhibition assay*. IgE cross-reactivity of house dust mite, cockroach, peanut and grass pollen extract tested by using an IgE ImmunoCAP inhibition assay. Bromelain, soluble egg antigens (SEA) of Schistosoma haematobium and Ascaris lumbricoides were used as inhibitors and values were expressed as the percentage of IgE inhibition.*

in 97 subjects but none of them showed any reactivity to 6 peanut components present on ImmunoCAP-ISAC. Of 27 subjects positive to whole extract of timothy grass pollen in CAP assay, 21 (77.7%) recognized nPhl p 4 on ImmunoCAP-ISAC. However, no reactivity was observed to the other 7 recombinant allergens of grass pollen (Figure 3d).

Association between helminth infection and IgE to allergen components and whole allergen extracts

Given the high prevalence of any helminth infection (93.2%) in our study subjects, it is not possible to determine the association of any helminth with allergen reactivity. However, the analysis of the association with specific helminths such as hookworm (76.3%), *A. lumbricoides* (42.1%) and *T. trichiura* (28.8%) was possible. We found that having *A. lumbriodes* was positively associated with IgE reactivity to rVes v 5 on ImmunoCAP-ISAC (OR, 3.42; 95% CI 1.22-9.59; p=0.019). No other associations were observed. No association between specific helminths and IgE in ImmunoCAP was seen.

DISCUSSION

To our knowledge, this is the first study to investigate IgE reactivity using an IgE microarray assay involving school children in an area in a developing country where helminth infections are highly endemic. We found strong IgE reactivity to natural component allergens from the pollen group, walnut and CCDs. Similarly, we found high reactivity to recombinant venom allergens (paper wasp, common wasp and honey bee). The reason for high reactivity to the pollen group and peanut appeared to be due to cross reactivity with the well-known CCD structures the α(1,3)-linked fucose and β(1,2)-linked xylose[34,35] which are carbohydrate components of plant and insect glycoproteins but also in helminths[36]. Little IgE reactivity was found to recombinant allergens of pollens. Importantly, for HDM and cockroach, the high reactivity, again was not directed to recombinant versions of well-established major allergens

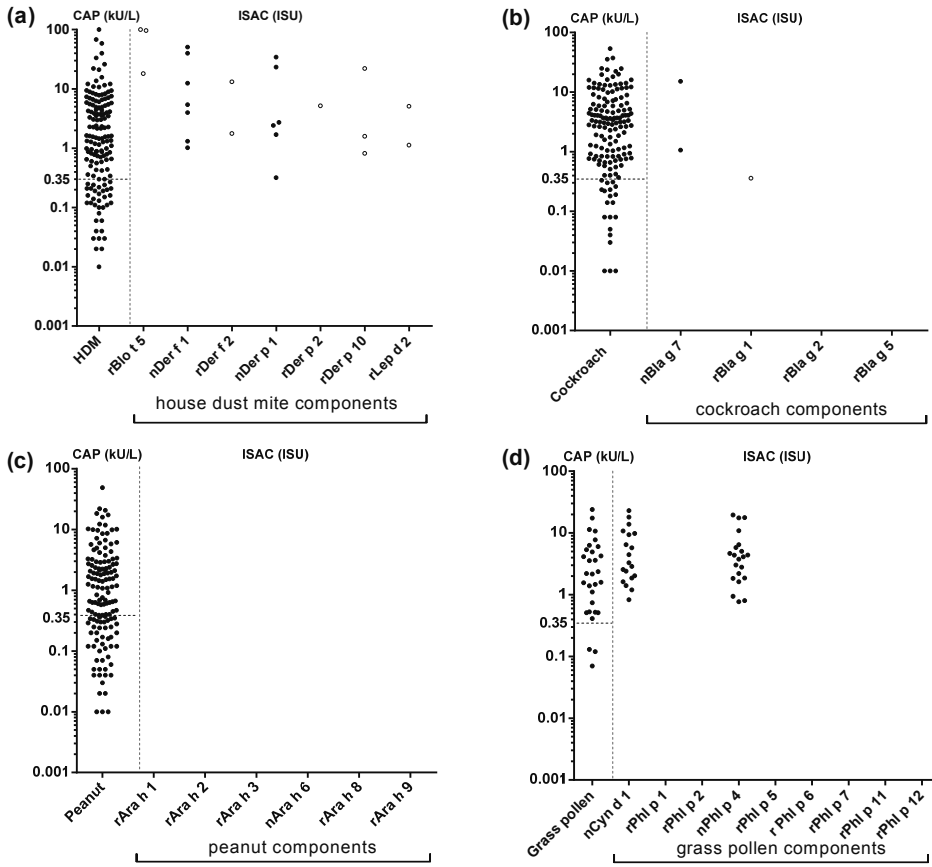


Figure 3. The levels of IgE to; (a) house dust mite, (b) cockroach, (c) peanut, and (d) grass pollen measured by ImmunoCAP and ImmunoCAP-ISAC. *The single allergens in ISAC are related to the whole allergen extracts tested in CAP. Solid circles represent natural (n) component allergens, open circles represent recombinant (r) component allergens.*

associated with primary sensitization. In this case IgE responses showed weaker associations to the typical CCD sources (MUXF3/bromelain), and more limited cross-reactivity in IgE inhibition. This leaves the question as to whether slightly different carbohydrate structures are responsible for sensitization and cross-reactivity or that other protein moieties thus far not present on the ISAC chip are involved. Since common allergens known to be associated with primary sensitization to house dust mite and cockroach were rarely recognized, sensitization to such "missing" allergens would most likely also be caused by other primary sources such as helminths. Some candidates have identified such as tropomyosin[22], but in our cohort this pan-allergen seems to be of limited importance (≤ 2% recognition). Other candidates not on the chip may be gluthation-S-transferases and paramyosins. As there is far less SPT or symptoms than IgE reactivity in this group studied, the data support the notion that in helminth endemic areas, IgE antibodies to a number of allergens arise form

cross reactivity between helminth derived or environmental carbohydrate structures and that these antibodies have poor functional properties.

Our results are in line with studies among subjects with and without allergic rhinitis in Philippines that showed an almost exclusive IgE reactivity to natural grass pollen allergens whereas non-glycosylated recombinant grass pollen allergens were hardly recognized[37]. Several studies in affluent countries have shown the existence of IgE antibodies directed to CCDs with negligible if any clinical relevance. Serum IgE from European pollen allergic patients cross-reacted with extracts from various allergenic foods which seemed to be tolerated[24]. Treating the extracts with periodate, which destroys the carbohydrate structures, abolished the reactions, indicating the involvement of carbohydrates in this cross reactivity[24]. Another study showed that elevated levels of IgE against peanut extract among grass pollen-sensitized European patients did not seem to translate to peanut SPT reactivity or clinical symptoms of peanut allergy[38]. In some of these patients, almost complete inhibition of IgE to peanut was possible with CCDs[38]. This was supported by another study, where about 42% of European pollen-allergic patients were found to have specific IgE to CCD marker bromelain without skin reactivity to this molecule[30]. The most convincing piece of evidence highlighting the lack of clinical relevance of CCD was provided by oral challenge with a purified glycoprotein polyvalently substituted with CCD[25].

In our study, we found that IgE against peanut as well as IgE to grass pollen were inhibited by bromelain (CCDs) which demonstrates that at least for pollen, anti-CCD IgE results in false-positive in vitro allergy test responses. Parasitic helminths carry carbohydrate structures which react with IgE. However, although bromelain could inhibit the ImmunoCAP of grass pollen and peanut, the extract of *A. lumbricoides,* which is endemic in our study area, was less potent. This indicates that either hookworm or *Trichuris* which are the other helminth species present in the study area might be the source of CCD. To support involvement of parasite-derived CCD, we used SEA from a non-endemic parasite that had been shown to be highly cross-reactive with peanut in a group of Ghanaian school children[16]. In the Ghanaian study, *A. lumbricoides* was also a poor inhibitor, as in our study. Using SEA, we found a strong inhibition of IgE to peanut and grass pollen. The characterization of the glycome of the endemic intestinal helminths should help us answer the question whether other cross reactive carbohydrate structures can explain the high IgE to peanut and grass pollen. The possibility that sources other than helminths in the rural area are responsible for the high cross reactive IgE seen in the area should also be studied further.

Although none of the study subjects showed IgE reactivity to natural bee venom allergen nApi m 4, we observed high IgE reactivity to recombinant venoms (rApi m 1, rPol d 5 or rVes v 5). However, why we find such high reactivity to venom allergens in children is not clear yet. Although it is possible that there is a true sensitization to venom allergens, it is also possible that the majority of positivity to honey bee/wasps represents sensitization to a peptide cross-reactive epitopes present on other insect proteins or helminths. We found that having *A. lumbricoides* increased the risk of IgE reactivity to rVes v 5, however, not to the other venom components. Therefore although the high venom IgE reactivity observed

in helminth endemic areas is not fully understood, it might be a cross-reactive response as a result of increased exposure to helminths which carry venom like proteins such as antigen 5. Although the clinical relevance of the high IgE to venoms needs to be formally investigated, there were no reports of clinical allergy to these allergens.

The strengths of this study include the microarray biochip which has allowed us to perform a comprehensive analysis of IgE reactivities to 112 component allergens from 51 source allergens, requiring only a small serum volume, which would have been impossible with traditional diagnostic tests. The ISAC chip is thus well suited where only small volumes of serum are available. However, a limitation of our study is that it was performed using a cross-sectional design in an area with no data on allergen exposure.

In conclusion, we provide evidence that there is cross-reactivity between IgE to allergens and CCD markers in a helminth-endemic area of Indonesia. Further epidemiological and clinical studies are needed to enable differentiation between IgE directed against protein structures that may be biologically active and IgE directed against carbohydrate moieties on glycosylated allergens that might be clinically irrelevant.

REFERENCES

1 Pawankar R, Canonica GW, et al. Introduction and Executive Summary: Allergic Diseases as a Global Public Health Issue In: Pawankar R, Canonica GW, Holgate S.T., LOckey RF, editors. World Allergy Organization (WAO) White Book on Allergy. 2011:11-20.

2 Bethony J, Brooker S, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521-32.

3 Yazdanbakhsh M, Kremsner PG, et al. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.

4 Flohr C, Quinnell RJ, et al. Do helminth parasites protect against atopy and allergic disease? Clin Exp Allergy 2009;39:20-32.

5 Feary J, Britton J, et al. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66:569-78.

6 Flohr C, Tuyen LN, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2010;40:131-42.

7 Cooper PJ, Chico ME, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006;367:1598-603.

8 Wiria AE, Hamid F, et al. The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: a household-based cluster-randomized, double-blind, placebo-controlled trial. PLoS One 2013;8:e57899.

9 Lynch NR, Hagel I, et al. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 1993;92:404-11.

10 Cooper PJ, Chico ME, et al. Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 2004;34:845-52.

11 Hamid F, Wiria AE, et al. Risk Factors Associated with the Development of Atopic Sensitization in Indonesia. PLoS One 2013;8:e67064.

12 Yazdanbakhsh M, van den Biggelaar A, et al. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001;22:372-7.

13 Wills-Karp M, Santeliz J, et al. The germless theory of allergic disease: revisiting the hygiene hypothesis. Nat Rev Immunol 2001;1:69-75.

14 van Ree R. Carbohydrate epitopes and their relevance for the diagnosis and treatment of allergic diseases. Int Arch Allergy Immunol 2002;129:189-97.

15 Acevedo N, Sanchez J, et al. IgE cross-reactivity between Ascaris and domestic mite allergens:

the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 2009;64:1635-43.

16 Amoah AS, Obeng BB, et al. Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol 2013; 132:639-47.

17 Nicolaou N, Poorafshar M, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. J Allergy Clin Immunol 2010;125:191-7.

18 Masthoff LJ, Mattsson L, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol 2013;132:393-9.

19 De Knop KJ, Bridts CH, et al. Component-resolved allergy diagnosis by microarray: potential, pitfalls, and prospects. Adv Clin Chem 2010;50:87-101.

20 Vieths S, Scheurer S, et al. Current understanding of cross-reactivity of food allergens and pollen. Ann N Y Acad Sci 2002;964:47-68.

21 Santos A, van Ree R. Profilins: mimickers of allergy or relevant allergens? Int Arch Allergy Immunol 2011;155:191-204.

22 Shanti KN, Martin BM, et al. Identification of tropomyosin as the major shrimp allergen and characterization of its IgE-binding epitopes. J Immunol 1993;151:5354-63.

23 Zuidmeer L, van Ree R. Lipid transfer protein allergy: primary food allergy or pollen/food syndrome in some cases. Curr Opin Allergy Clin Immunol 2007;7:269-73.

24 Aalberse RC, Koshte V, et al. Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 1981;68:356-64.

25 Mari A, Ooievaar-de HP, et al. Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. Allergy 2008;63:891-6.

26 van Die, I, Gomord V, et al. Core alpha1-->3-fucose is a common modification of N-glycans in parasitic helminths and constitutes an important epitope for IgE from Haemonchus contortus infected sheep. FEBS Lett 1999;463:189-93.

27 Hiller R, Laffer S, et al. Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment. FASEB J 2002;16:414-6.

28 Passalacqua G, Melioli G, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. Allergy 2013;68:1029-33.

29 Scala E, Alessandri C, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Clin Exp Allergy 2010;40:911-21.

30 Mari A, Iacovacci P, et al. Specific IgE to cross-reactive carbohydrate determinants strongly affect the in vitro diagnosis of allergic diseases. J Allergy Clin Immunol 1999;103:1005-11.

31 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol 2002;129:286-95.

32 Wiria AE, Prasetyani MA, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2010;10:77.

33 Hamid F, Wiria AE, et al. A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis 2011;11:83.

34 Aalberse RC, Akkerdaas J, et al. Cross-reactivity of IgE antibodies to allergens. Allergy 2001;56:478-90.

35 van Ree R, Cabanes-Macheteau M, et al. Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J Biol Chem 2000;275:11451-8.

36 Altmann F. The role of protein glycosylation in allergy. Int Arch Allergy Immunol 2007;142:99-115.

37 Cabauatan CR, Lupinek C, et al. Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates. J Allergy Clin Immunol 2014;133:910-4.

38 van der Veen MJ, van Ree R, et al. Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 1997;100:327-34.

SUPPLEMENTARY TABLES

Table S1. The prevalence of sensitization on ImmunoCAP-ISAC

Component allergens	N (%)	Component allergens	N (%)
Food components		*Cockroach*	
Shrimp (nPen m 2)	2 (1.3)	Cockroach (rBla g 1)	1 (0.7)
Shrimp (nPen m 4)	3 (2.0)	*Venom*	
Walnut (nJug r 2)	48 (32.0)	Honey bee (rApi m 1)	28 (18.7)
Soybean (nGly m 5)	2 (1.3)	Paper wasp (rPol d 5)	55 (36.7)
Soybean (nGly m 6)	1 (0.7)	Common wasp (rVes v 5)	51 (34.0)
Wheat (rTri a 19.0101)	1 (0.7)	*Latex*	
Grass pollen		Latex (rHev b 5)	2 (1.3)
Bermuda grass (nCyn d 1)	76 (50.7)	*Cross-reactive*	
Timothy grass (rPhl p 1)	1 (0.7)	Tropomyosin	
Timothy grass (nPhl p 4)	80 (53.3)	Anisakis (rAni s 3)	1 (0.7)
Tree pollen		Cockroach (nBla g 7)	2 (1.3)
Japanese cedar (nCry j 1)	28 (18.7)	*D. pteronyssinus* (rDer p 10)	3 (2.0)
Cypress (nCup a 1)	32 (21.3)	Shrimp (nPen m 1)	2 (1.3)
Olive pollen (nOle e 1)	13 (8.7)	nsLTP	
Olive pollen (rOle e 9)	1 (0.7)	Walnut (nJug r 3)	2 (1.3)
Plane tree (rPla a 1)	19 (12.7)	Peach (rPru p 3)	2 (1.3)
Plane tree (nPla a 2)	46 (30.7)	Plane tree (rPla a 3)	1 (0.7)
Weed pollen		PR-10 protein	
Plantain (rPla l 1)	1 (0.7)	Hazel pollen (rCor a 1.0101)	3 (2.0)
Saltwort (nSal k 1)	1 (0.7)	Hazelnut (rCor a 1.0401)	4 (2.7)
Animal		Apple (rMal d 1)	4 (2.7)
Dog (rCanf2)	1 (0.7)	Peach (rPru p 1)	5 (3.3)
Mold		Kiwi (rAct d 8)	2 (1.3)
Alternaria (rAlt a 6)	2 (1.3)	CCD	
Aspergillus (rAsp f 1)	1 (0.7)	nMUXF3	32 (21.3)
Aspergillus (rAsp f 3)	3 (2.0)		
Cladosporium (rCla h 8)	1 (0.7)		
Mite			
Blomia tropicalis (rBlo t 5)	3 (2.0)		
Dermatophagoides farinae (nDer f 1)	7 (4.7)		
D. farinae (rDer f 2)	2 (1.3)		
D. pteronyssinus (nDer p 1)	6 (4.0)		
D. pteronyssinus (rDer p 2)	1 (0.7)		
Lepidoglyphus destructor (rLep d 2)	2 (1.3)		

Natural (n) and recombinant (r) allergen components. The number positive (N) of 150 subjects examined.

SUPPLEMENTARY FIGURES

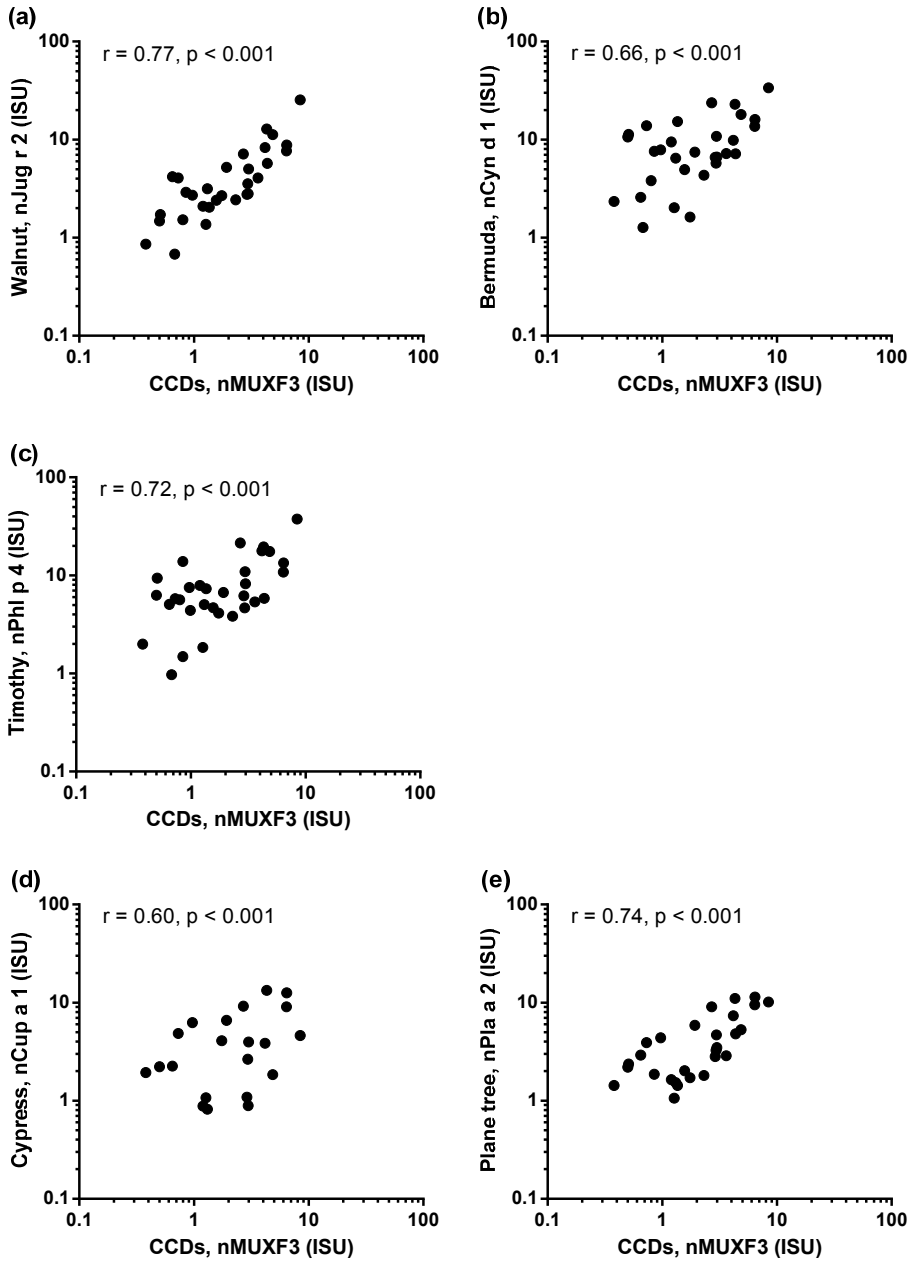


Figure S1. Correlation between IgE to CCD and natural allergens on ImmunoCAP-ISAC; *(a) walnut, (b) bermuda, (c) timothy, (d) cypress, and (e) plane tree. nMUXF3 is a marker glycoprotein for CCD reactivity on ISAC assay. r = correlation coefficient. ISU, ISAC Standardized Unit.*

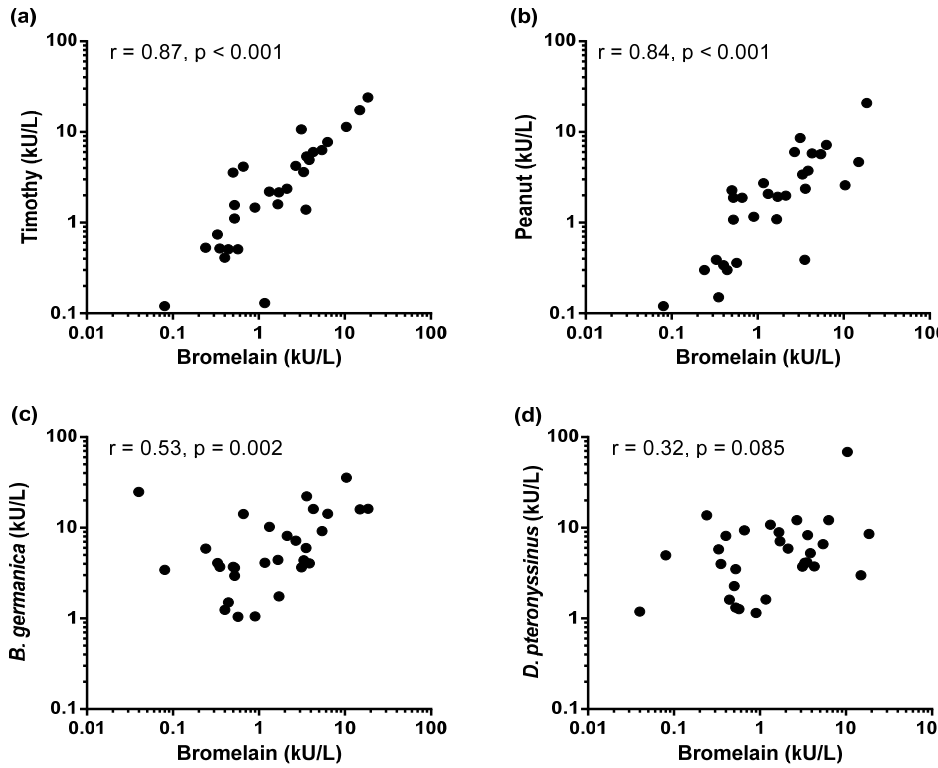


Figure S2. Correlation between IgE levels to bromelain and allergens on ImmunoCAP; *(a) timothy, (b) peanut, (c) cockroach, and (d) house dust mite. Bromelain is a marker glycoprotein for CCD reactivity. r = correlation coefficient.*

7 SUMMARIZING DISCUSSION

Partly based on:
Helminth-induced IgE and protection against allergic disorders

Firdaus Hamid[1,2], Abena S Amoah[2,3], Ronald van Ree[4], Maria Yazdanbakhsh[2]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana
4. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Current Topics in Microbiology and Immunology 2015; 388: 91-108

WHAT WAS ALREADY KNOWN ABOUT THE RELATIONSHIP BETWEEN HELMINTH INFECTIONS, SOCIO-ECONOMIC STATUS AND ALLERGIC DISORDERS?

It has been reported in several epidemiological studies that the prevalence of atopic disorders is higher in developed countries than in developing countries[1,2]. In contrast, intestinal helminth infections such as *Ascaris lumbricoides, Trichuris trichiura* and/or hookworms (*Necator americanus* or *Ancylostoma duodenale*) are still highly prevalent in developing countries[3]. Interestingly, both helminth infection and allergens are potent inducers of Th2 responses that lead to high levels of IgE, tissue eosinophilia, as well as the secretion of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13[4,5]. Despite the similar immunological profiles associated with both helminth and allergies, the relation between helminth infections and atopic diseases is still unclear. Several studies have reported an inverse relation between helminth infections and atopic diseases[6-8] while other studies have shown that helminth infections might increase the risk of asthma or be associated with higher prevalence of atopy[9] (reviewed in **Chapter 1**). Interventional studies have also not been consistent; one large study in Ecuador found no changes in allergic disorders after one year treatment of intestinal helminths using albendazole[10], which is in contrast to an intervention study in Vietnamese children that suggested repeated anthelmintic treatment might increase the incidence of atopy[11].

Several studies have shown conflicting results with regards to the role of socio-economic status (SES) in the development of atopic disorders[12]. However, a recent systematic review has suggested that lower socio-economic levels are associated with higher prevalence of asthma, whereas the prevalence of other allergies such as food allergy and atopic dermatitis is associated with higher SES[12]. This systematic review might coffer from emphasis on data from Europe and the Americas, as well as data from high- and middle-income countries. Moreover, skin prick test (SPT) reactivity to any allergens has been reported to be more common in high-SES groups compared to low-SES groups within urban centers of developing countries[13,1].

In both developed and developing countries, a strong correlation is observed between allergen-specific IgE and symptoms of allergy among urban populations of high SES. However, in rural populations or urban population of developing countries with low SES, helminth-induced IgE cross-reactivity and regulatory networks may prevent the translation of allergen-specific IgE into skin reactivity or allergic symptoms as discussed in **Chapter 1**.

In addition, the isolation and characterization of allergen components, as well as their production by recombinant techniques, has led to significant progress in allergy diagnosis[15]. Component-resolved diagnostics (CRD) allows the detection of specific IgE against individual allergen molecules instead of against allergen extracts comprised of mixtures of allergen molecules that are commonly used in SPT and conventional specific IgE testing. The advantage of CRD is that distinction can be made between IgE antibodies against allergenic structures with different degrees of clinical relevance, ranging from irrelevant such as cross-reactive carbohydrate determinants (CCD)[16] to a risk factor for severe symptoms[17]. This

SUMMARY OF WHAT WAS ALREADY KNOWN

- There is an inverse geographical relationship between allergy and helminth infections.
- The prevalence of atopy and allergic diseases is increasing in both developed and developing countries.
- Low socio-economic status is associated with asthma symptoms and negatively with skin prick test (SPT) reactivity to any allergens.
- Anthelmintic treatment increases SPT in Vietnam but not in Ecuador.
- High levels of total IgE and allergen-specific IgE do translate into skin reactivity or clinical symptoms of allergy in most affluent countries.
- Component-resolved diagnosis of the specific IgE responses improves the specificity, sensitivity and clinical performance of laboratory assays used in the diagnosis of atopic disorders in developed countries.

method has been widely used in developed countries[15], but has still not been widely used in less affluent areas where helminth infections are highly prevalent.

HOW DID OUR STUDIES ADVANCE THE FIELD?

In order to move the field further, we designed a population study on Flores Island in Indonesia, detailed in **Chapter 3**.

Risk factors for atopic disorders and interactions with socio-economic status

In **Chapter 2 and 4**, evidence was provided that socio-economic status is an important factor in atopic disorders in Indonesia. We found that high parental education, which is one of the indicators of high-SES, was associated with skin reactivity to house dust mite (HDM) (**Chapter 4**). In addition, we also found that the prevalence of skin prick test reactivity to any allergens was higher in high-SES compared to low-SES schoolchildren (**Chapter 2**) or higher in a semi urban compared to a rural area (**Chapter 4).** This result is in line with other studies conducted in Ghanaian children, which found that the prevalence of SPT to any allergens was higher in urban rich compared to urban poor children[13,14]. We also observed that there was a strong association between specific IgE and SPT reactivity to the same aeroallergen in the children from a high-SES school (**Chapter 2**), a result that is consistent with what is found in developed countries[18,19]. However, no such association was seen in the low-SES group (**Chapter 2**) living in the same urban area. Similar dissociation between specific IgE (sIgE) to aeroallergens and skin prick test reactivity to the same allergen has been reported by us in a rural area of Indonesia (**Chapter 4).** The prevalence of allergic symptoms was higher in low-SES children than in high-SES children which is in contrast to what is often reported[20,21]. This might be due to the inability to distinguish between symptoms that arise

due to respiratory infections rather than allergy. Indeed, we observed that SPT positivity is a risk factor for reported symptoms of allergy in high-SES but not in low-SES children (**Chapter 2**). This result implies that SPT can be used as a good predictor for allergy symptoms in high-SES children, comparable to the situation in developed countries, but not for children of low-SES. Altogether, the high-SES life-style which is associated with western diet and less physical activity may contribute towards atopy compared to low-SES environments.

Environmental factors are closely related to socio-economic status. One of the important environmental factors is thought to be helminth infection. In **Chapter 2 and 4** we provide evidence that helminth infections are more prevalent among low SES groups compared to high SES groups. Important contextual determinants for helminth infection are poverty, lack of sanitation and poor hygiene practices in low-SES population[22]. With regard to the association between helminth infections and atopic disorders, in a meta-analyses, Feary *et al.* have shown that hookworm infections determined by microscopy are significantly associated with lower SPT reactivity to HDM[23]. In chapter 4, we used a highly sensitive and specific method (*polymerase chain reaction*, PCR) for detection of soil transmitted helminth infections and were able to demonstrate that high load of hookworm infection decreased the risk of being prick test positive to HDM in the semi-urban area only (**Chapter 4**). However, the situation was different when SPT to cockroach was examined. In the same semi-urban area, children with access to piped water coming from non-farming families had reduced risk of SPT to cockroach (**Chapter 4**). The precise cause of this association for skin reactivity to cockroach is unknown but it is thought to be related to higher exposure to this aeroallergen in low-SES houses, which might defy the mechanisms that are responsible for the lower prevalence of SPT to HDM. However, it is also possible that different mechanisms govern HDM versus cockroach SPT reactivity.

In summary, within the same urban centres of a developing country; our study found that depending on the level of SES of a child, the prevalence of different allergy outcomes or risk factors for atopic disorders may be very different. Our study also indicates that the lower prevalence of skin reactivity to HDM in a rural area of Indonesia might be due to a lower level of education (as part of the SES indicators) and a greater degree of helminth infections compared with the semi urban area. Moreover, the risk factors for sensitization to HDM are distinct from those for sensitization to cockroach. Together, these results highlight that environmental as well as socio-economic factors should be considered by clinicians and researchers working on prevention, diagnosis and treatment of atopic disorders in low-to-middle income countries. In addition, many factors in the environment contribute to the development of allergies (e.g. diet, immunisation, pets, tobacco smoke, and air pollution), therefore, more research is needed to evaluate the possible risk and protective factors in more detail as well as to pinpoint elements of SES that matter in the development of atopic disorders.

Effect of anthelmintic treatment on atopic disorders

Considering that cross sectional studies can only show relationships and do not demonstrate causality between helminths and allergies, interventional studies where anthelmintics are

used to remove helminths or intentional infection with helminths are needed. With respect to anthelmintic treatment, a study of soil transmitted helminth infected subjects demonstrated that regular anthelmintic treatment resulted in significant increase in skin test reactivity as well as IgE in serum to aeroallergens[24]. In line with this, anthelmintic treatment of Gabonese children chronically infected with *Schistosoma haematobium* and soil transmitted helminths resulted in increased SPT reactivity[7]. Two large interventional studies previously mentioned conducted in Ecuador and Vietnam showed different results. In Ecuador, treatment with albendazole every two months for one year, did not affect SPT nor clinical symptoms of allergy[10], but in Vietnam, three monthly treatment with albendazole resulted in a significant increase in SPT positivity but not in allergy symptoms[11]. In **Chapter 5**, our randomised placebo controlled trial showed that intensive community treatment of 3 monthly albendazole for 21 months was not associated with increased risk of SPT to any allergen but post hoc analysis showed that SPT to cockroach allergen was increased in the albendazole arm compared to placebo. However, in agreement with the studies in Ecuador and Vietnam, there was no effect on reported clinical symptoms of allergy (**Chapter 5**). It has to be noted that one study in Venezuela showed that anthelmintic treatment resulted in improvement in all clinical indicators of asthma[25]. It is important to mention that in our study, helminth infections were not completely cleared; therefore the effect to anthelmintic treatment on allergy outcome could not be fully seen. A study in Ecuador, looking at communities receiving anthelmintic treatment with ivermectin for 15-17 years compared to non-treated communities showed an increase in the prevalence of allergen skin test reactivity in treated communities[26]. This suggests that longer anthelmintic treatment in our study may have been needed to see the effect of treatment on atopic disorders. When considering anthelmintic treatment given during pregnancy, a large randomized, double-blind, placebo-controlled trial carried out in Uganda found that treatment of pregnant women with albendazole (compared with placebo) was strongly linked to an increased risk of doctor-diagnosed infantile eczema in their infants[27].

Taken together, most studies, but not all, show that helminth infections are associated with decreased SPT but there does not seem to be a strong effect on clinical symptoms with the possible exception of a beneficial effect on infantile eczema. For anthelmintic treatment studies, it is possible that different helminths with their varying life cycles and locations in tissues, would lead to different effects on allergic outcomes. Moreover, it should be noted that chronicity of infection as well as worm burden might be important parameters to take into account when studying the association between helminths and allergies. Chronic infections as well as higher worm burdens might have stronger regulatory effect on allergies than acute or light infections[28]. Another mechanism that might explain the inverse association between helminth infections and atopy may involve helminth-induced IgE cross-reactivity. Current helminth infections are associated with increased levels of allergen-specific IgE that do not translate into skin reactivity or clinical symptoms. This helminth-induced IgE may be of low affinity; this might explain why it does not lead to SPT reactivity. Finally, attention should be paid to the methods used to assess clinical symptoms of allergy as these could be an important source of variation.

Microarray and Helminth-induced IgE

In recent years, microarray biochips have been developed to allow the simultaneous measurement of specific IgE to multiple recombinant and natural allergen components using a small amount of serum. These microarray biochips are increasingly used in developed countries to provide additional information on IgE profiles of poly-sensitized allergic patients to improve the management of their conditions[15,29,30].

In a group of children (described in chapter 4), high levels of IgE to house dust mite were found, but these did not translate into skin prick test reactivity. In this study, helminth-induced IgE cross-reactivity was implicated as a possible explanation for the elevated levels of clinically irrelevant allergen-specific IgE. In a subset of these children, the specific IgE to *Dermatophagoides pteronyssinus* (Der p) determined by the ImmunoCAP method (sensitization cut-off ≥ 0.35 kU_A/L) was compared to semi-quantitative IgE analysis using a commercially available microarray chip (ImmunoCAP-ISAC). We found that the prevalence of IgE sensitization to whole house dust mite extract (Der p) was 74% while sensitization to purified recombinant and natural house dust mite allergens such as Der p 1 and Der p 2 as assessed by the microarray biochip was generally weak and only up to 5% (**Chapter 6**). This investigation also demonstrated that among these same children, there was IgE reactivity to purified natural (glycosylated) allergens of Bermuda and Timothy grass pollen but not to (non-glycosylated) recombinant grass pollen allergens on the chip (**Chapter 6**). As shown in Figure 1, the microarray technique used on a serum sample from a helminth-infected individual compared to a European allergic patient can differentiate between IgE directed against protein structures that may be biologically active and IgE directed against carbohydrate moieties on glycosylated allergens (such as natural glycosylated pollen allergens) that have been shown to be clinically irrelevant. Essentially, helminth-infected Indonesian children recognize purified natural pollen allergens because of having carbohydrate-specific IgE antibodies but do not recognize non-glycosylated recombinant pollen (and house dust mite) allergens. Helminth-induced cross-reactivity is most likely at the basis of these IgE responses. European children on the other hand clearly react with the latter category of allergens supporting true sensitization. Also, the high prevalence of positive IgE to peanut in the Indonesian children was shown to be caused by IgE against CCD, as demonstrated by inhibition with bromelain (a commonly used marker glycoprotein for CCD reactivity) and SEA (soluble egg antigen of *S. haematobium,* a source of helminth-derived CCD). The commonly recognized major peanut allergens (Ara h 1, 2, 3 and 6) were not recognized.

As described in **Chapter 6**, we also found high reactivity to recombinant venom allergens among school children in Indonesia. This cannot be explained by CCD. The source of sensitization to these venom allergens in children who do not appear to have clinical venom allergy is yet not clear. This unexpected IgE detection can be explained in two possible ways; there is a true sensitization to some kind of venom allergens (not necessarily the common wasps or bees represented by the recombinant allergens on the chip), or the majority of cases of double positivity to honey bee/wasp allergens may be due to sensitization to cross-reactive proteins of non-insect venom origin (e.g. of helminths).

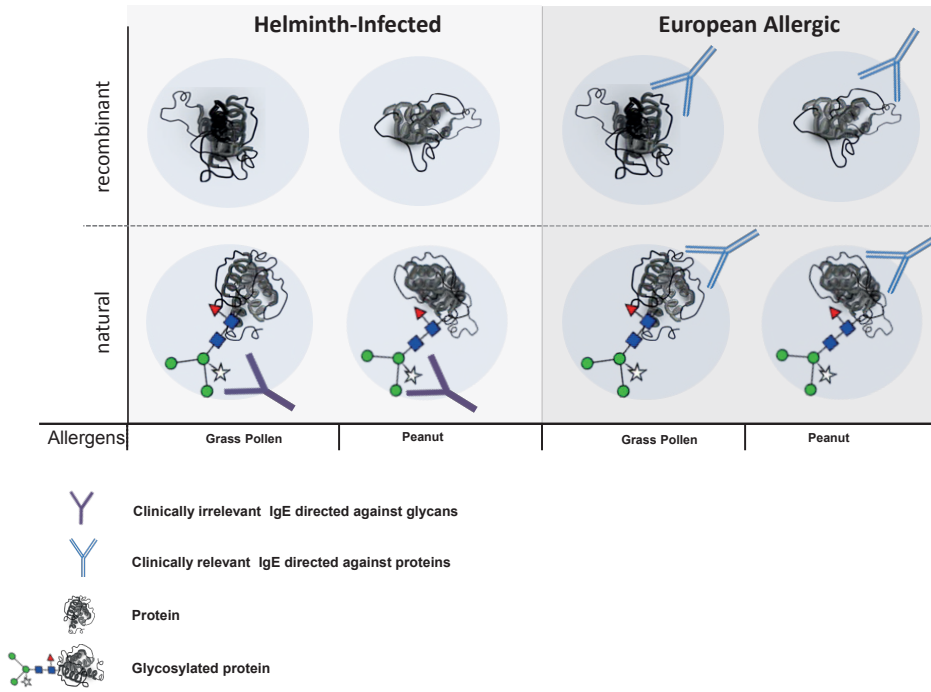


Figure 1. Determination of allergic sensitization by microarray. *An illustration of typical component-resolved diagnosis results generated using the ImmunoCAP ISAC™ microarray. The microarray shown contains natural and recombinant grass pollen as well as natural and recombinant peanut allergens. For an allergic European, the IgE recognizes and binds to the protein structures on both the natural and recombinant allergens for both grass pollen and peanut. This IgE is biologically active and also clinically relevant. In the serum of a helminth infected subject, elevated levels of IgE are observed that recognize and bind to carbohydrate moieties on the natural allergen components. This IgE does not bind to the protein structures of these components, is clinically irrelevant, and shows poor biological activity.*

In summary, the study presented on the profile of IgE antibodies using a biochip array and plasma from a helminth-endemic area has provided evidence that there is a strong IgE reactivity to some of the natural allergen components from pollen which carry CCDs. This reactivity is probably helminth-induced. Our study also found unexpectedly high reactivity to recombinant venom allergens in children from a helminth-endemic area, but the origin of this sensitization still needs to be elucidated. We provide evidence of cross-reactivity between IgE to allergens and CCD markers, suggesting that using natural and recombinant allergens on microarray might help to better differentiate between real allergic sensitization and cross-reactivity for diagnosis of atopic disorders in low-to-middle income countries where helminth infections are prevalent. Further research into helminths and IgE should focus on refining and preparing new diagnostic methods for the developing world where allergies are increasing but the diagnosis is hampered by the lack of knowledge on locally important allergen sources and the complexity of the specificities and characteristics of IgE antibodies. Furthermore, a better understanding of how IgE cross-reactivity develops and how this affects the biological activity of the antibody is needed.

SUMMARY OF THE FINDINGS

In this thesis, we investigated the risk factors for atopic sensitization and allergic disease in some areas of Indonesia where the socio-economic status and life style are very different from affluent countries. We studied the effect of anthelmintic treatment on atopic disorders. Furthermore, we characterized the IgE antibody profiles among children who are living in a helminth-endemic area. The main findings are:

- Specific IgE was a risk factor for being SPT positive and SPT positivity was a major risk factor for reported clinical symptoms of allergy in high-SES children from an urban area but not in low-SES (**Chapter 2**).
- High load of hookworm (*Necator americanus*) infection based on PCR method was an independent protective factor for HDM skin reactivity (**Chapter 4**)
- Cockroach skin reactivity was increased in children from farmer families, which are presumed to have higher exposure to cockroach (**Chapter 4**)
- Repeated three-monthly treatment with single dose albendazole over 21 months reduced but did not eliminate helminth infection (**Chapter 5**)
- Two years anthelminthic treatment increased skin reactivity especially to cockroach (**Chapter 5**).
- In an helminth-endemic area, carbohydrate-bearing allergens were more recognized than recombinant allergen components (**Chapter 6**)
- High reactivity to recombinant venom allergen was observed in children from a helminth-endemic area (**Chapter 6**)

REFERENCES

1 Pawankar R, Canonica GW, et al. Introduction and Executive Summary: Allergic Diseases as a Global Public Health Issue. In: Pawankar R, Canonica GW, Holgate S.T., LOckey RF, editors. World Allergy Organization (WAO) White Book on Allergy. 2011:11-20.

2 Weinmayr G, Weiland SK, et al. Atopic sensitization and the international variation of asthma symptom prevalence in children. Am J Respir Crit Care Med 2007;176:565-74.

3 Bethony J, Brooker S, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 2006;367:1521-32.

4 Yazdanbakhsh M, van den Biggelaar A, et al. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001;22:372-7.

5 Yazdanbakhsh M, Kremsner PG, et al. Allergy, parasites, and the hygiene hypothesis. Science 2002;296:490-4.

6 Lynch NR, Hagel IA, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol 1998;101:217-21.

7 van den Biggelaar AH, van Ree R, et al. Decreased atopy in children infected with Schistosoma haematobium: a role for parasite-induced interleukin-10. Lancet 2000;356:1723-7.

8 Dagoye D, Bekele Z, et al. Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am J Respir Crit Care Med 2003;167:1369-73.

9 Palmer LJ, Celedon JC, et al. Ascaris lumbricoides infection is associated with increased risk of

childhood asthma and atopy in rural China. Am J Respir Crit Care Med 2002;165:1489-93.

10 Cooper PJ, Chico ME, et al. Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 2006;367:1598-603.

11 Flohr C, Tuyen LN, et al. Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 2010;40:131-42.

12 Uphoff E, Cabieses B, et al. A systematic review of socioeconomic position in relation to asthma and allergic diseases. Eur Respir J 2014.

13 Addo Yobo EO, Custovic A, et al. Exercise induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. Thorax 1997;52:161-5.

14 Obeng BB, Amoah AS, et al. Schistosome infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian schoolchildren. Clin Exp Allergy 2014;44:965-75.

15 De Knop KJ, Bridts CH, et al. Component-resolved allergy diagnosis by microarray: potential, pitfalls, and prospects. Adv Clin Chem 2010;50:87-101.

16 Mari A, Ooievaar-de HP, et al. Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. Allergy 2008;63:891-6.

17 Masthoff LJ, Mattsson L, et al. Sensitization to Cor a 9 and Cor a 14 is highly specific for a hazelnut allergy with objective symptoms in Dutch children and adults. J Allergy Clin Immunol 2013;132:393-9.

18 Ollert M, Weissenbacher S, et al. Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing. Clin Chem 2005;51:1241-9.

19 Ro AD, Saunes M, et al. Agreement of specific IgE and skin prick test in an unselected cohort of two-year-old children. Eur J Pediatr 2012;171:479-84.

20 Poyser MA, Nelson H, et al. Socioeconomic deprivation and asthma prevalence and severity in young adolescents. Eur Respir J 2002;19:892-8.

21 Mercer MJ, Joubert G, et al. Socioeconomic status and prevalence of allergic rhinitis and atopic eczema symptoms in young adolescents. Pediatr Allergy Immunol 2004;15:234-41.

22 Vandemark LM, Jia TW, et al. Social science implications for control of helminth infections in Southeast Asia. Adv Parasitol 2010;73:137-70.

23 Feary J, Britton J, et al. Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66:569-78.

24 Lynch NR, Hagel I, et al. Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 1993;92:404-11.

25 Lynch NR, Palenque M, et al. Clinical improvement of asthma after anthelminthic treatment in a tropical situation. Am J Respir Crit Care Med 1997;156:50-4.

26 Endara P, Vaca M, et al. Long-term periodic anthelmintic treatments are associated with increased allergen skin reactivity. Clin Exp Allergy 2010;40:1669-77.

27 Mpairwe H, Webb EL, et al. Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 2011;22:305-12.

28 Smits HH, Hammad H, et al. Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol 2007;120:932-40.

29 Valenta R, Lidholm J, et al. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 1999;29:896-904.

30 Gadisseur R, Chapelle JP, et al. A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP© 250 with the ImmunoCAP© ISAC. Clin Chem Lab Med 2011;49:277-80.

APPENDIX

SUMMARY

There has been a global increase in prevalence of allergic diseases. This is particularly so in developed and in urban centres of developing countries. An inverse geographical pattern is seen when one considers helminth infections. Exposure to helminth infections has been minimal in developed countries and is being controlled in urban centres of developing countries due to improvement in sanitation and education as well as improved medical care. At the same time, in rural areas of developing countries helminth infections are still highly prevalent.

Interestingly, both helminth infection and allergens are potent inducers of Th2 responses that lead to high levels of IgE, eosinophils, as well as the secretion of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13. Despite the similar immunological profiles associated with both helminth and allergies, several epidemiological studies have failed to show a consistent relationship between helminth infections and allergies. The role of socio-economic status (SES) which can be intertwined with presence or absence of helminth infections, has been examined in relation to development of allergic disorders in several studies. The results are gain conflicting.

In both developed and developing countries, a strong correlation is observed between allergen-specific IgE and symptoms of allergy among urban populations of high SES. However, in developing countries, in rural populations or urban populations with low SES, helminth-induced IgE cross-reactivity and regulatory networks may prevent the translation of allergen-specific IgE into skin reactivity or allergic symptoms.

In addition, the isolation and characterization of allergen components, as well as their production by recombinant techniques, has led to significant progress in allergy diagnosis. Component-resolved diagnostics (CRD) allow the detection of specific IgE against individual allergen molecules instead of against allergen extracts comprised of mixtures of allergen molecules, which are commonly used in SPT and conventional specific IgE testing. The CRD method has been widely used in developed countries, but less used in non-affluent areas where helminth infections are highly prevalent.

The studies presented in this thesis shed light on the relationship between helminth infection, SES and atopic disorders in a developing country (Indonesia) where large differences in life-style, environmental exposure and SES are seen.

Chapter 1

This chapter presents the associations found between helminth infections and atopic disorders from previous studies. It also discusses how helminth infection can lead to IgE cross-reactivity with allergens and how this IgE has poor biological activity. In addition, it describes important information regarding the use of new diagnostic methods using CRD for allergic disorders in countries where helminth infections are highly prevalent.

Chapters 2 and 4

Recent studies indicate that helminth infection is one of the environmental factors that, together with socio-economic status (SES), contribute to the development of allergies. In Chapters 2 and 4 we examined differences in prevalence of allergic outcomes in rural and semi-urban areas of Indonesia on Flores Island and in children of high and low SES families from an urban centre. Higher prevalence of skin reactivity to house dust mite (HDM) was found in the semi-urban area that seemed to be due to higher level of education (as part of the SES indicators) and a lower degree of helminth infections compared to the rural area. When considering children from the same urban centre but belonging to families with different socio-economic status, we found that whereas specific IgE to HDM increased the risk of being skin prick test positive and that a positive SPT to HDM was then associated with increased risk of wheezing in children of high SES, this was not the case in children of low SES families. Taken together, these results highlight that environmental as well as socio-economic factors should be considered by clinicians and researchers working on prevention, diagnosis and treatment of atopic disorders in low-to-middle income countries. More research is needed to evaluate the possible risk and protective factors in more detail as well as to pinpoint elements of SES that matter in the development of atopic disorders.

Chapter 3

Here we provide a study protocol of a household randomized placebo-controlled trial in Flores Island to answer the question whether helminth infections play a role in the development of atopic disorders. This chapter describes in detail the overview of the study population in a semi-urban and a rural area of Flores Island, Indonesia, which is used in chapters 4, 5 and 6.

Chapter 5

This chapter reports the malarial parasitemia and allergy outcomes of a 2-years duration randomized trial. Our two-year placebo controlled deworming trial shows a trend that deworming increases skin reactivity to any allergen. We found a significant increase in SPT reactivity to cockroach after 21 months of anthelmintic treatment. In addition, we also found that repeated three-monthly treatment with albendazole reduces but does not eliminate helminth infection. These findings suggest that longer intensive anthelmintic treatment, possibly a combination of drugs, or drugs and environmental control, is needed for future studies to achieve helminth elimination.

Chapter 6

The study presented here, regarding the profile of IgE antibodies using a biochip array and plasma from a helminth-endemic area, provides evidence that there is strong IgE reactivity to some of the natural allergen components from pollen which carry cross-reactive carbohydrate determinants (CCDs). This reactivity is probably helminth-induced. Our study also found

unexpectedly high reactivity to recombinant venom allergens (bee, common wasp and paper wasp) in children from a helminth-endemic area, but the origin of this sensitization still needs to be elucidated. We provide evidence of cross-reactivity between IgE to allergens and CCD markers, suggesting that using natural and recombinant allergens on the microarray might help to better differentiate between primary sensitization that is biologically relevant and cross-reactivity for diagnosis of atopic disorders in helminth-endemic low-to-middle income countries. Further research into helminths and IgE should focus on refining and preparing new diagnostic methods for the developing world where allergies are increasing but the diagnosis is hampered by the lack of knowledge on locally important allergen sources and the complexity of the specificities and characteristics of IgE antibodies.

Chapter 7

This chapter summarizes and discusses the main findings of the thesis. Taken together, we have demonstrated the association between atopic disorders and helminth infection and SES, as well as provided evidence that two years of deworming has a minimal impact on allergy outcomes. We characterized IgE antibody profiles in a helminth-endemic area and we showed that high levels of IgE do not translate to skin prick test reactivity or clinical symptoms of allergy.

&

SAMENVATTING

De prevalentie van allergische aandoeningen neemt wereldwijd toe, zowel in de Westerse wereld als in stedelijke gebieden in ontwikkelingslanden. De geografische verspreiding van helminth-infecties laat een omgekeerd patroon zien: blootstelling aan wormen is minimaal in Westerse landen en wordt in de steden van ontwikkelingslanden verminderd door maatregelen op het gebied van hygiëne, voorlichting en medische voorzieningen. Op het platteland van ontwikkelingslanden is de prevalentie van helminth-infecties echter nog hoog.

Interessant genoeg induceren zowel worminfecties als allergenen een sterke Th2-immuunreactie, die bestaat uit hoge IgE-concentraties, eosinofilie en de uitscheiding van Th2-cytokines zoals IL-4, IL-5, IL-9 en IL-13. Ondanks het vergelijkbare immunologische profiel van helminth-infecties en allergieën, komt uit epidemiologische onderzoeken en interventiestudies geen eenduidige relatie tussen beide naar voren. Het hebben van een helminth-infectie is gedeeltelijk vervlochten met sociaal-economische status (SES). Uit verschillende onderzoeken naar de rol die SES speelt bij het ontwikkelen van allergieën komen wisselende resultaten naar voren.

In zowel ontwikkelde als ontwikkelingslanden wordt er een sterke correlatie gevonden tussen allergeen-specifiek IgE en allergische symptomen in stadspopulaties met een hoge SES. Deze correlatie gaat in ontwikkelingslanden echter niet op voor plattelandsbewoners en stedelingen met lage SES, hetgeen mogelijk verklaard wordt door worm-geassocieerde IgE-kruisreactiviteit en immunoregulatoire netwerken die de progressie van allergeen-specifiek IgE naar huidreacties of allergische symptomen voorkomen.

De isolatie, karakterisatie en productie van allergeencomponenten met behulp van recombinante technieken heeft geleid tot belangrijke verbeteringen binnen de diagnostiek van allergieën. 'Component-resolved diagnostics' (CRD) maakt het mogelijk om specifiek IgE te detecteren dat gericht is tegen individuele allergenen, in plaats van tegen een mix van allergeen-extracten zoals gebruikt wordt bij huidpriktesten (skin prick test, SPT) en conventionele methoden om specifiek IgE te detecteren. De CRD-methode wordt veel gebruikt in de Westerse wereld, maar minder in ontwikkelingslanden, waar worminfecties veelvuldig voorkomen.

De onderzoeken in dit proefschrift bekijken de relatie tussen worminfecties, SES en atopische aandoeningen in een ontwikkelingsland (Indonesië), waar grote verschillen bestaan in levensstijl, blootstelling aan omgevingsfactoren en SES.

Hoofdstuk 1

Dit hoofdstuk vat gegevens uit voorgaande studies samen over de associatie tussen worminfecties en atopische aandoeningen. Tevens wordt hierin besproken hoe worminfecties kunnen leiden tot IgE-kruisreactiviteit met allergenen en het feit dat dit IgE slechts matige biologische activiteit heeft. Verder wordt belangrijke informatie beschreven betreffende het gebruik van CRD beschreven als nieuwe diagnostische methode voor allergische aandoeningen in landen waar veel worminfecties voorkomen.

Hoofdstukken 2 en 4

Recent onderzoek heeft uitgewezen dat helminth-infectie één van de omgevingsfactoren is die samen met SES bijdraagt aan het ontstaan van allergieën. In hoofdstukken 2 en 4 belichten we de verschillen in prevalentie van allergieën, zowel tussen het platteland en semi-stedelijke gebied op het eiland Flores (Indonesië) als tussen stedelijke kinderen met lage en hoge SES. Ten opzichte van het platteland bleek in semi-stedelijk gebied een hogere prevalentie van huidreacties tegen huisstofmijt, waarschijnlijk veroorzaakt door een hoger opleidingsniveau (onderdeel van de SES) en minder helminth-infecties. Wanneer we kinderen van verschillende SES binnen dezelfde stad vergeleken, bleek dat alleen in kinderen met een hoge SES de kans op een piepende ademhaling voorspeld werd door een positieve huidtest tegen huisstofmijt, hetgeen op zichzelf geassocieerd was met het hebben van specifiek IgE tegen huisstofmijt. Beide verbanden gingen niet op voor kinderen met een lage SES. Samenvattend geven deze resultaten aan dat zowel omgevings- als socioeconomische factoren in acht moeten worden genomen door artsen en onderzoekers die werken op het gebied van preventie, diagnostiek en behandeling van atopische aandoeningen in laag- en midden-inkomenslanden. Er is meer onderzoek nodig om de mogelijke risico- en beschermende factoren die betrokken zijn bij het ontstaan van atopische aandoeningen in meer detail uit te zoeken en om te achterhalen welke onderdelen van de SES hierin de belangrijkste rol spelen.

&

Hoofdstuk 3

Hier wordt het studieprotocol weergegeven van een placebo-gecontroleerde trial gerandomiseerd op huishouden op het eiland Flores, om de vraag te beantwoorden of worminfecties een rol spelen bij de ontwikkeling van atopische aandoeningen. Dit hoofdstuk beschrijft gedetailleerd de onderzoekspopulaties in een semi-stedelijk en een plattelandsgebied op het eiland Flores (Indonesië), die gebruikt zijn in de hoofdstukken 4, 5 en 6.

Hoofdstuk 5

Dit hoofdstuk beschrijft de resultaten ten aanzien van malaria-parasitemie en allergie-prevalentie van het twee jaar durende veldonderzoek. Onze trial van twee jaar placebo-gecontroleerd ontwormen laat zien dat het behandelen van wormen de allergische huidreactiviteit licht verhoogd. Er was een significante toename van huidreactiviteit tegen kakkerlak-componenten na 21 maanden ontwormen in vergelijking met placebo. Verder bleek dat herhaalde behandeling met albendazol elke drie maanden de hoeveelheid worminfecties vermindert maar niet geheel elimineert. Dit suggereert dat langere en intensievere wormbehandeling, mogelijk met een combinatie van middelen, nodig is bij toekomstig onderzoek naar de effecten van het elimineren van wormen.

Hoofdstuk 6

Het onderzoek in hoofdstuk 6, waarin het profiel van IgE-antilichamen werd bepaald met een biochip-array in plasmamonsters uit een gebied endemisch voor wormen, levert bewijs dat er sterke IgE-reactiviteit is tegen sommige componenten van pollen die kruisreagerende koolhydraatdeterminanten (cross-reactive carbohydrate determinants, CCDs) bij zich dragen. Deze reactiviteit wordt waarschijnlijk veroorzaakt door worminfecties. Onze studie heeft ook onverwacht hoge reactiviteit gevonden tegen recombinante toxines van bijen en wespen in kinderen uit een wormrijk gebied, maar de oorsprong van deze reactiviteit moet nog verder worden onderzocht. We laten zien dat er kruisreactiviteit bestaat tussen IgE tegen allergenen en koolhydraatdeterminanten. Dit suggereert dat het gebruik van natuurlijke en recombinante allergenen op de microarray in helminth-endemische ontwikkelingslanden zou kunnen leiden tot een beter onderscheid tussen kruisreactiviteit en primaire sensitisatie die werkelijk biologisch relevant is. Verder onderzoek naar wormen en IgE zou zich moeten richten op nieuwe en meer verfijnde diagnostische methoden voor ontwikkelingslanden. Dit is van belang omdat allergieën steeds meer voorkomen in ontwikkelingslanden maar de diagnostiek wordt gehinderd door gebrek aan kennis over lokaal voorkomende allergenen en de complexe eigenschappen van IgE-antilichamen.

Hoofdstuk 7

In dit hoofdstuk worden de belangrijkste bevindingen van dit proefschrift samengevat en bediscussieerd. We hebben aangetoond hoe worminfecties en SES een rol spelen bij het ontstaan en de ontwikkeling van atopische aandoeningen en we hebben bewijs geleverd dat twee jaar ontwormen een minimale impact heeft op allergie. We hebben IgE-antilichamen verder gekarakteriseerd in een wormrijk gebied en we hebben laten zien dat hoge IgE-concentraties niet altijd leiden tot huidreactiviteit of andere klinische symptomen van allergie.

CURRICULUM VITAE

Firdaus Hamid was born in Ujung Pandang, Indonesia on the 31st of December 1977. He completed his medical degree in June 2002 in the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. After completing his medical degree, he worked at the Department of Microbiology in the same faculty where he was appointed as a lecturer in December 2002. Thereafter, he was engaged in *Salmonella typhi* and dengue research under the supervision of Prof. dr. Moch. Hatta, Dr. Rizalinda Sjahril, Dr. Isra Wahid and Prof. dr. Muh. Nasrum Massi. In 2006, he was selected as a PhD candidate for a collaborative project between Hasanuddin University (UNHAS), University of Indonesia UI, Jakarta and Leiden University Medical Center (LUMC) funded by the Royal Netherlands Academy of Arts and Sciences (KNAW). The results of this collaborative project (a longitudinal randomized controlled anthelminthic trial) form the basis of this thesis. As part of his PhD, he had to start on a new network topic, set up surveys and a field laboratory in a region with limited infrastructure on Flores Island, Indonesia. He was supervised by Professor Taniawati Supali from UI and Dr. Sitti Wahyuni from UNHAS for the work conducted on Flores Island. In 2010, he travelled to the Department of Parasitology at LUMC to work under supervision of Dr. Erliyani Sartono and Professor Maria Yazdanbakhsh towards completing his PhD thesis. He received a European Academy of Allergy and Clinical Immunology Exchange Research Fellowship in 2012. This allowed him to conduct additional research in the Department of Experimental Immunology, Academic Medical Center, University of Amsterdam, The Netherlands, under the supervision of Professor Ronald van Ree. This resulted in chapter 6 of this thesis. After completion of his PhD studies, he will return to his post of lecturer at UNHAS and will conduct research, in addition to taking on the responsibility for the medical curriculum as he was recently appointed as the coordinator of the medical education unit.

LIST OF PUBLICATIONS

Hamid F, Versteeg SA, Wiria AE, Wammes LJ, Wahyuni S, Supali T, Sartono E, van Ree R*, Yazdanbakhsh M*. *Molecular diagnostics in a helminth-endemic area in Indonesia provides leads for the lack of clinical allergy. Manuscript in submission*

Wammes LJ*, **Hamid F***, Wiria AE*, May L, Kaisar MM, Prasetyani MA, Djuardi Y, Wibowo H, Kruize YC, Verweij JJ, de Jong SE, Tsonaka R, Houwing-Duistermaat JJ, Sartono E, Luty AJ, Supali T, Yazdanbakhsh M. *Community deworming alleviates geohelminth-induced immune hyporesponsiveness: a household-clustered randomised controlled trial. Manuscript in submission*

Wiria AE, **Hamid F**, Wammes LJ, Prasetyani MA, Dekker OM, May L, Kaisar MM, Verweij J, Guigas B, Partono F, Sartono E, Supali T, Yazdanbakhsh M, Smit JW. *Infection with soil-transmitted helminths is associated with increased insulin sensitivity.* PLoS One. 2015;10:e0127746.

Hamid F, Wahyuni S, van Leeuwen A, van Ree R, Yazdanbakhsh M, Sartono E. *Allergic disorders and socio-economic status: a study of school children in an urban area of Makassar, Indonesia.* Clin Exp Allergy. 2015;7:1226-36.

Hamid F, Amoah AS, van Ree R, Yazdanbakhsh M. Helminth-induced IgE and protection against allergic disorders. Curr Top Microbiol Immunol. 2015;388:91-108.

Amoah AS, **Hamid F**, Smits HH, Yazdanbakhsh M. *Environmental risk factors for allergy: helminth infections.* In C. A. Akdis & I. Agache (Eds.), Global Atlas of Allergy. Published by the European Academy of Allergy and Clinical Immunology. 2014:138-140.

Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Djuardi Y, Versteeg SA, Wahyuni S, van Ree R, Sartono E, Supali T, Yazdanbakhsh M. *Risk Factors Associated with the Development of Atopic Sensitization in Indonesia.* PLoS One. 2013;8:e67064.

Kaisar MM, Supali T, Wiria AE, **Hamid F**, Wammes LJ, Sartono E, Luty AJ, Brienen EA, Yazdanbakhsh M, Lieshout LV, Verweij JJ. *Epidemiology of Plasmodium infections in Flores Island, Indonesia using real-time PCR.* Malar J. 2013;12:169.

Wiria AE*, **Hamid F***, Wammes LJ*, Kaisar MM, May L, Prasetyani MA, Wahyuni S, Djuardi Y, Ariawan I, Wibowo H, Lell B, Sauerwein R, Brice GT, Sutanto I, van Lieshout L, de Craen AJ, van Ree R, Verweij JJ, Tsonaka R, Houwing-Duistermaat JJ, Luty AJ, Sartono E, Supali T, Yazdanbakhsh M. *The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: a household-based cluster-randomized, double-blind, placebo-controlled trial.* PLoS One. 2013;8:e57899.

Wammes LJ, Wiria AE, Toenhake CG, **Hamid F**, Liu KY, Suryani H, Kaisar MM, Verweij JJ, Sartono E, Supali T, Smits HH, Luty AJ, Yazdanbakhsh M. *Asymptomatic Plasmodial Infection Is Associated With Increased Tumor Necrosis Factor Receptor II-Expressing Regulatory T Cells and Suppressed Type 2 Immune Responses.* J Infect Dis. 2013;207:1590-9.

**these authors contributed equally*

Wiria AE, Wammes LJ, **Hamid F**, Dekkers OM, Prasetyani MA, May L, Kaisar MM, Verweij JJ, Tamsma JT, Partono F, Sartono E, Supali T, Yazdanbakhsh M, Smit JW. *Relationship between carotid intima media thickness and helminth infections on Flores Island, Indonesia.* PLoS One. 2013;8:e54855.

Wammes LJ, **Hamid F**, Wiria AE, Wibowo H, Sartono E, Maizels RM, Smits HH, Supali T, Yazdanbakhsh M. *Regulatory T cells in human lymphatic filariasis: stronger functional activity in microfilaremics.* PLoS Negl Trop Dis. 2012;6:e1655.

Hamid F, Wiria AE, Wammes LJ, Kaisar MM, Lell B, Ariawan I, Uh HW, Wibowo H, Djuardi Y, Wahyuni S, Schot R, Verweij JJ, van Ree R, May L, Sartono E, Yazdanbakhsh M, Supali T. *A longitudinal study of allergy and intestinal helminth infections in semi urban and rural areas of Flores, Indonesia (ImmunoSPIN Study).* BMC Infect Dis. 2011;11:83.

Supali T, Verweij JJ, Wiria AE, Djuardi Y, **Hamid F**, Kaisar MM, Wammes LJ, van Lieshout L, Luty AJ, Sartono E, Yazdanbakhsh M. *Polyparasitism and its impact on the immune system.* Int J Parasitol. 2010;40:1171-6.

Wiria AE, Prasetyani MA, **Hamid F**, Wammes LJ, Lell B, Ariawan I, Uh HW, Wibowo H, Djuardi Y, Wahyuni S, Sutanto I, May L, Luty AJ, Verweij JJ, Sartono E, Yazdanbakhsh M, Supali T. *Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study).* BMC Infect Dis. 2010;10:77.

Wammes LJ, **Hamid F**, Wiria AE, de Gier B, Sartono E, Maizels RM, Luty AJ, Fillié Y, Brice GT, Supali T, Smits HH, Yazdanbakhsh M. *Regulatory T cells in human geohelminth infection suppress immune responses to BCG and Plasmodium falciparum.* Eur J Immunol. 2010;40:437-42.

ACKNOWLEDGMENTS

This thesis would not have been written without supervision, assistance, goodwill and help of many individuals. I would like to extend my sincere thanks to all of the following people:

Supervisors
Maria Yazdanbakhsh
Ronald van Ree
Erliyani Sartono
Taniawati Supali
Sitti Wahyuni

Paranymphs
Aprilianto Eddy Wiria
Linda J Wammes

Scientific input/mentors
Adrian J Luty
Agus Rachmat
Bertrand Lell
Cornelis H Hokke
Franca Hartgers
Hae Won Uh
Heri Wibowo
Hermelijn Smits
Iwan Ariawan
Jaco J Verweij
Jeanine Houwing-Duistermaat
Lisette van Lieshout
Linda May
Roula Tsonaka
Serge A Versteeg
Yenny Djuardi

Parasitology FKUI
Erlinda Marthias
Heni Suryani
Intan Razari
Margaretta Prasetyani
Romauli Tryana Sinaga
Sovie Nurlinda
Stefanus Dening Arytansu
Sudirman

Nangapanda teams
Helda Sihotang & family
Ical, Ima, Markus, Sensi
Karolina Sona & family
Puskesmas Nangapanda
Puskesmas Welamosa

All study participants & field workers

LUMC PARA colleagues
Abena S Amoah
Yvonne Kruize
Aldian Irma Amaruddin
Alwin van der Ham
Angela van Diepen
Anouk Gloudemans
Arifa Ozir-Fazalalikhan
Astrid L Voskamp
Ayola Akim Adegnika
Bart Everts
Beatrice M F Winkel
Bruno Guigas
Cornelis H Smit
Caroline Remmerswaal
Corrie Verbree
Dicky Tahapary
D. Linh Nguyen
Elias Asuming Brempong
Eric A T Brienen
Ferry Heus
Jacqueline Janse
Jantien Guldemond
Honorine Lima Mbenkep
Karin de Ruiter
Katja Obieglo
Kit Yeng Liu
Leonard R Plegrom
Leonie Hussaarts
Luciën van der Vlugt
Łucja Łabuda
Lynn Meurs
Maria M M Kaisar
Marijke C C Langenberg
Michelle Y Yang
Moustapha Mbow
M Eunice Betouke Ongwe
Nicole Driessen
Noemí Garcíoa Tardón
Sanne de Jong
Simone Häberlein
Sunny Sapthu
Ulysse Ateba Ngoa
William van der Puije
Yoanne D Mouwenda
Yolanda van Wijck
all the other PARA's

Doctorate & Opposition Committees

FK UNHAS
Andi Asadul Islam
Asaad Maidin
Baedah Madjid
Irawan Yusuf
Moch Hatta
Muh Nasrum Massi
Rizalinda Sjahril
Syafri K Arif

Other colleagues and friends
Abbas Makkasau & family
Andi Rofian Sultan
Andi Ahmad Yani
Andriany Qanitha
Awal Setiawan
Dedi Sjamsuddin & family
Dessy Rachmawati
Dianawaty Amiruddin
Eli ten Lohuis
Emile Jonker
Ivonne Martin
Jac Reus
Malik Batubara
Muh Pandu Samudero
Muh Ruslin
Nurhayana A Sennang
Syamsuar M
Sunarti Tutu Heersink & family
Yanky Ong
Wahid Nadji
Wendy Sugianto
Yayan Sundara
Yusuf Wibisono

Family
Mama' Nurlena & Etta Hamid
My wife Sari
My children Firsa & Hana
Mama Darmawanti & Bapak Burham
Lutfi-Ida, Nurham-Ajis, Ida-Ali
Lukman-Tina, Zulham-Yus
Zulfah-Abos, Qudri-Muli
Kurnia-Ramlah, Dewi-Ferry
Tatok-Dian, Tony-Niken

&