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).

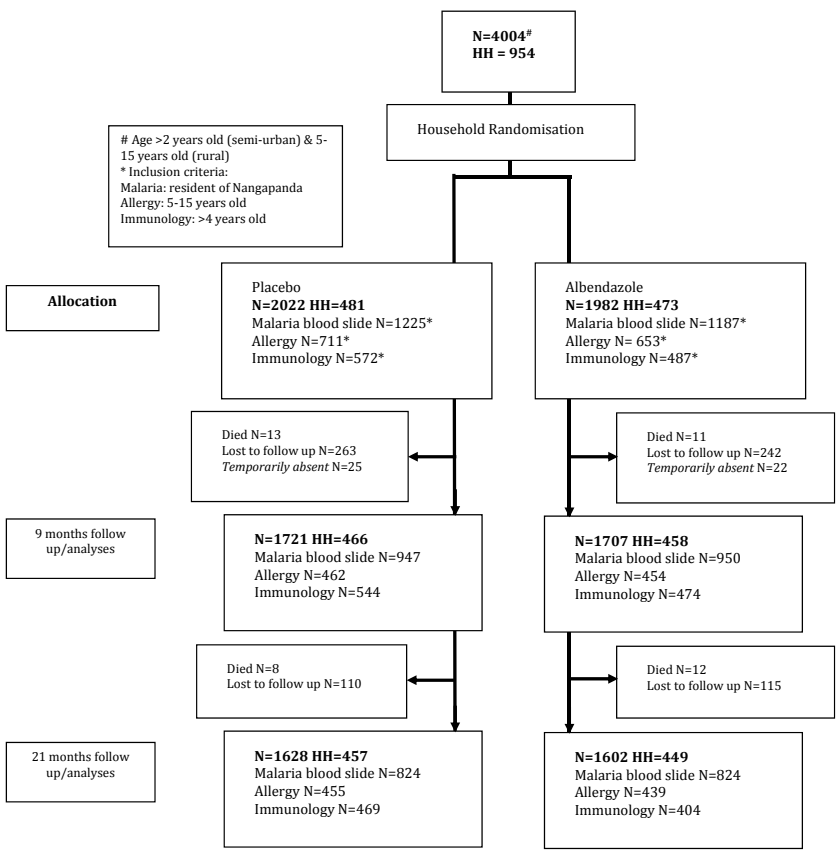


Figure 1. Trial Profile. *HH: Household. Lost to follow up implies that the participants have no data from this time point onward. Temporarily absent implies that the participants have no data at this time point but have data available at other time point*

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)

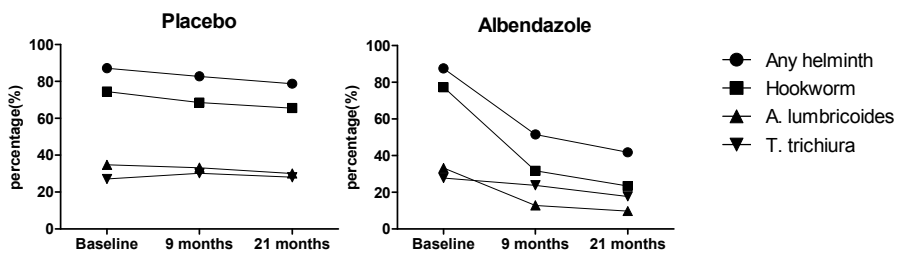
[1]diagnosed by PCR; [2]diagnosed by microscopy. The number of positives (n) of the total population examined (N)

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

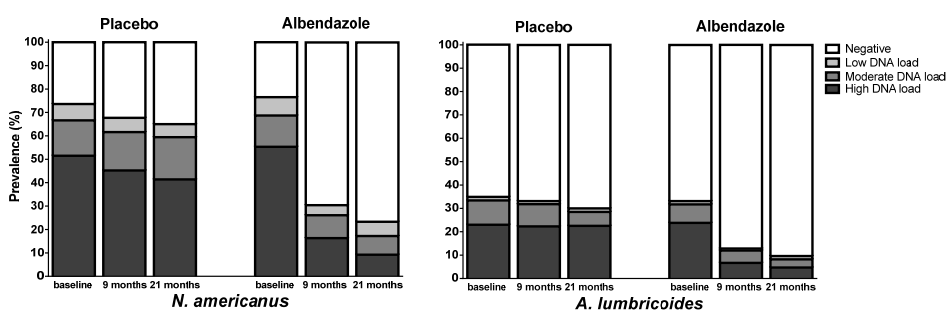


Figure 2. Effect of treatment on helminth prevalence and intensity. *A) Percentage of helminth infected subjects in placebo and albendazole treatment arms. The presence of hookworms (by PCR), Ascaris lumbricoides (by PCR) and Trichuris trichiura (by microscopy) or any of these helminth infections in 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).

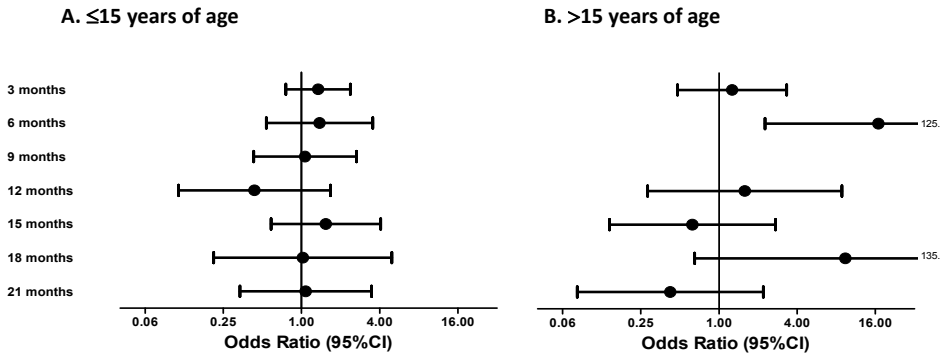


Figure 3. Effect of albendazole treatment on malarial parasitemia based on two age categories. *Malarial parasitemia A) ≤15 and B) >15 years of age. The risk of malarial parasitemia after albendazole treatment compared to placebo is shown as odds ratio with 95% CI. The reference line is set at 1, indicating 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

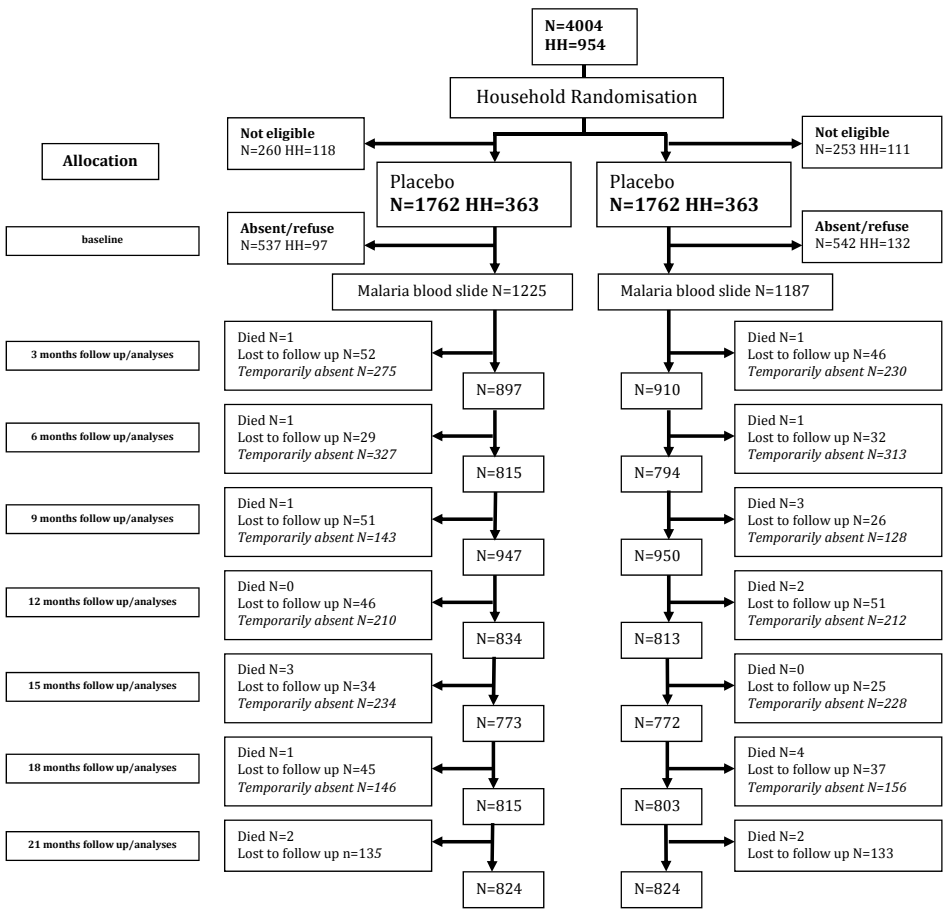


Figure S1A. Profile of trial with malarial parasitemia as outcome in the village of Nangapanda where malaria is endemic

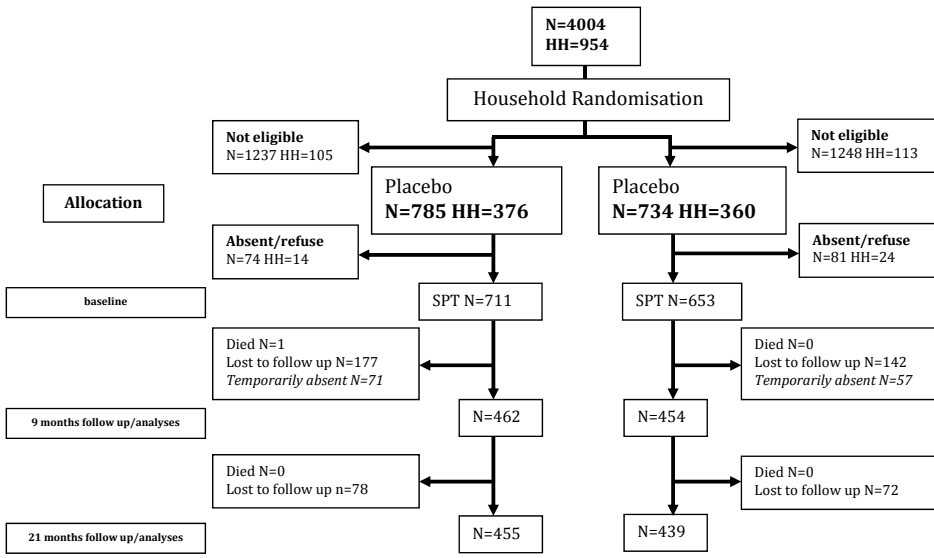


Figure S1B. Profile of trial with skin prick test (SPT) reactivity as outcome in children 5-15 years of age in both Nangapanda and Anaranda

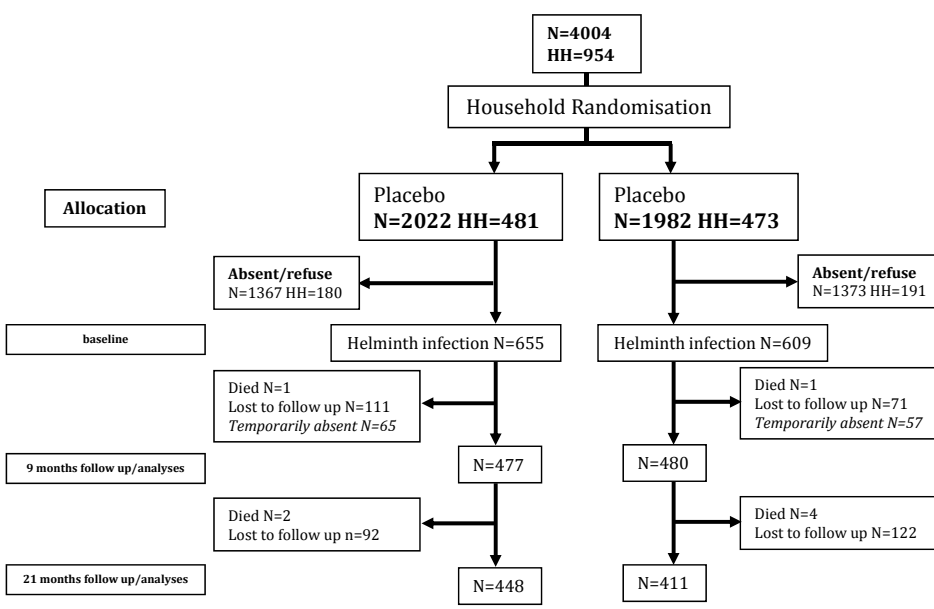


Figure S1C. Profile of trial with helminth infection as outcome in villages of Nangapanda and Anaranda