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The Effect of Three-Monthly Albendazole Treatment on Malarial Parasitemia and Allergy: A Household-Based Cluster-Randomized, Double-Blind, Placebo-Controlled Trial

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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 and Findings: 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. 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

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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

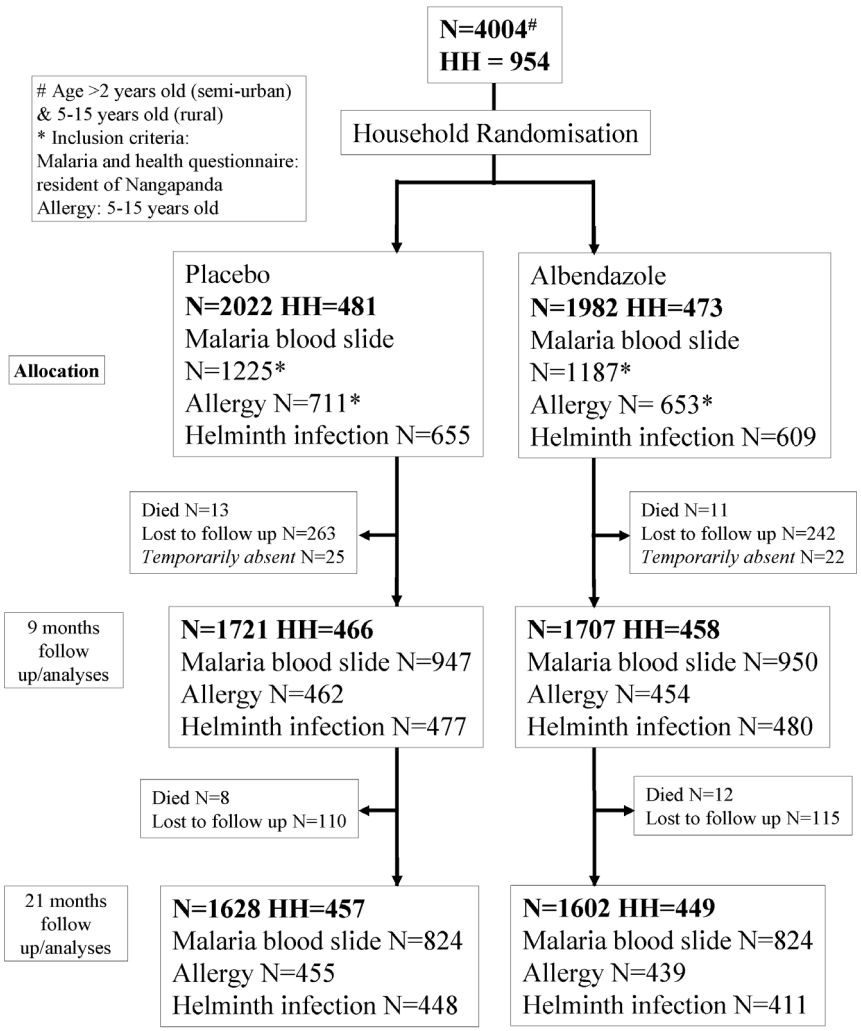

Figure 1. Trial Profile. HH: Household. Lost to follow up implies that the participants have no data from this time point onward. Temporarily absent implies that the participants have no data at this time point but have data available at other time point.
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with 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. The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1.

Randomization and masking

The population was randomized by 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).

Table 1. Baseline characteristics.

	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/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).
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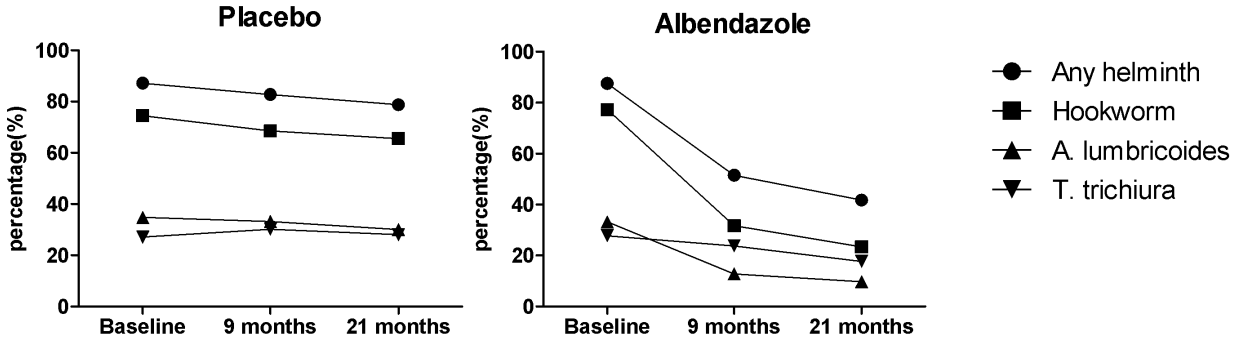
Procedures

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*. (Appendix S1, p2), 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 (*Blatella 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*

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

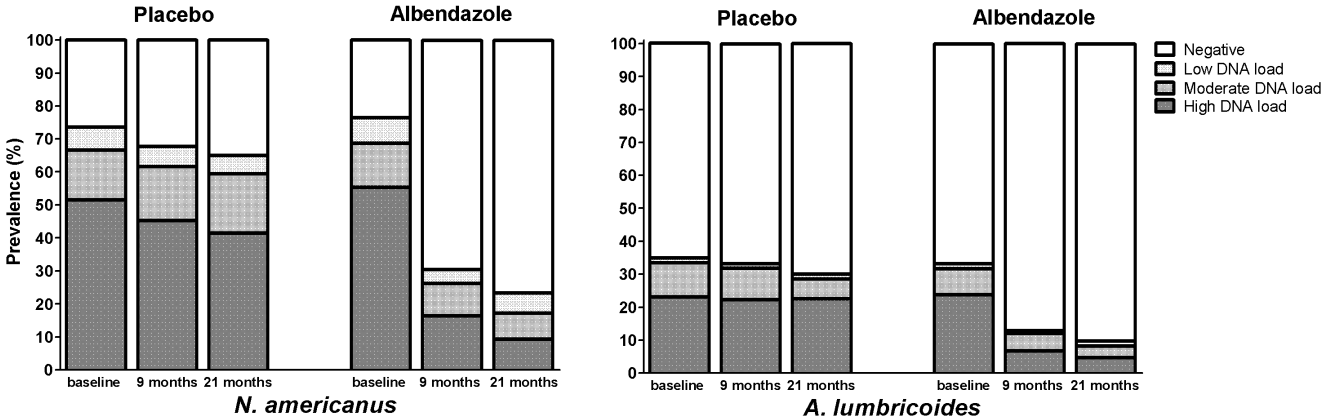


Figure 2. A) Percentage of helminth infected subjects in placebo and albendazole treatment arms. The presence of hookworms (by PCR), *Ascaris lumbricoides* (by PCR) and *Trichuris trichiura* (by microscopy) or any of these helminth infections in subjects who provided stool samples at baseline, 9 and 21 months post treatment (numbers are given in table S1A in Appendix S1). **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.
doi:10.1371/journal.pone.0057899.g002

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] (Appendix S1, p2). 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

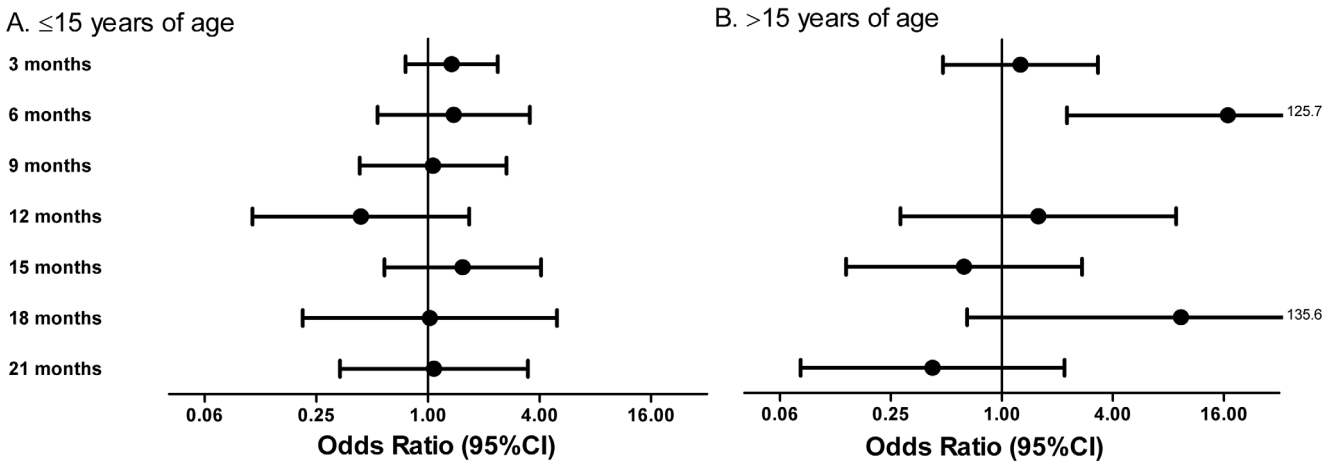


Figure 3. Effect of albendazole treatment on malarial parasitemia based on two age categories. Malarial parasitemia A) ≤15 and B) >15 years of age. The risk of malarial parasitemia after albendazole treatment compared to placebo is shown as odds ratio with 95% CI. The reference line is set at 1, indicating 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 ∞.
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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 are 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 (Appendix S1, 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). At baseline 87·3% of the individuals were infected with one or more helminth species. The baseline

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

	P. falciparum		*P. vivax*		*P. malariae*	
	Placebo	**Albendazole**	**Placebo**	**Albendazole**	**Placebo**	**Albendazole**
	n/N (%)	**n/N (%)**	**n/N (%)**	**n/N (%)**	**n/N (%)**	**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).
doi:10.1371/journal.pone.0057899.t002

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

	Placebo	Albendazole	OR (95%CI) *
	n/N (%)	n/N (%)	
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.
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characteristics were similar between the treatment arms (table 1). The overall trial profile is shown in figure 1, and figure S1A, S1B, S1C (p13–15) in Appendix S1 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 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.

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

	Placebo	Albendazole	OR (95% CI)
	n/N (%)	n/N (%)	
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).
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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 Appendix S1 p8). 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. 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 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). 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 in Appendix S1 p10).

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 (1.63 (1.07–2.50)) (table 6). The levels of IgE to allergens showed that albendazole treatment had no effect (table 6). No effect of treatment was seen on symptoms of asthma or atopic dermatitis (table S3 in Appendix S1 p11).

No significant change in BMI was observed in children or in adults (table S4 in Appendix S1 p12). 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 be taken as an incomplete reduction. The results show a transient increase in

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

	Placebo	Albendazole	OR (95%CI) *
	n/N (%)	n/N (%)	
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).
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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 anthelminthic treatment have also reported conflicting results.[29,30] A small study conducted in Madagascar[29] has reported an increase in malarial parasitemia in levimasole treated subjects, older than 5 years of age, while in Nigeria [30], 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. 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 years 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

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

	Placebo	Albendazole	
Skin prick test reactivity*	**n/N (%)**	**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).
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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 spesies 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 hyporesponsiveness 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 a 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 *Ascaris 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.

Supporting Information

Appendix S1 Here we describe additional details on methods: study area and procedure, data collection on clinical symptoms, and detailed description of statistical models used. We provide tables on the effect of three monthly albendazole treatment on helminth infections, on reported fever and malaria-like symptoms, on reported clinical symptoms of allergy, and on body mass index. In addition, trial profiles of the separate outcomes are provided: malarial parasitemia, skin prick test, and helminth infection.
(DOC)

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial Protocol.
(DOC)

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Author Contributions

Contributed to the setting up the field study: GTB IS. Led the work on PCR detection of parasites: JJV LL. Contributed to parasitological investigation: MMMK. Did the randomization: IA. Contributed to safeguarding randomization codes and privacy of the study subjects: HW. Contributed to the setting up of the data base and monitoring: BL LM. Advised the allergy study: SW. Conceived and designed the experiments: MY TS. Performed the experiments: AEW FH LJW. Analyzed the data: MY JJH RT AJFL AEW FH LJW LM YD ES TS. Contributed reagents/materials/analysis tools: RR RS. Wrote the paper: MY JJH AJMC AJFL AEW FH LJW LM YD ES TS.

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Supplementary Methods

Additional information on the study area and procedures

Ende district, an area highly endemic for STH, is situated near the equator (8^{o}45'S, 121^{o}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 95ºC followed by 50 cycles of 15 s at 95ºC, 30 s at 60ºC, and 30 s at 72ºC. 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^{o}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 (≥37.5, 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		Unadjusted	Adjusted
	Events (PM)	Incidence per PM	Events (PM)	Incidence per PM	IRR	IRR
Fever	414 (18494)	0.02	429 (18636)	0.02	1.03	1.03
Headache	333 (19067)	0.02	340 (19563)	0.02	1.00	1.00
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

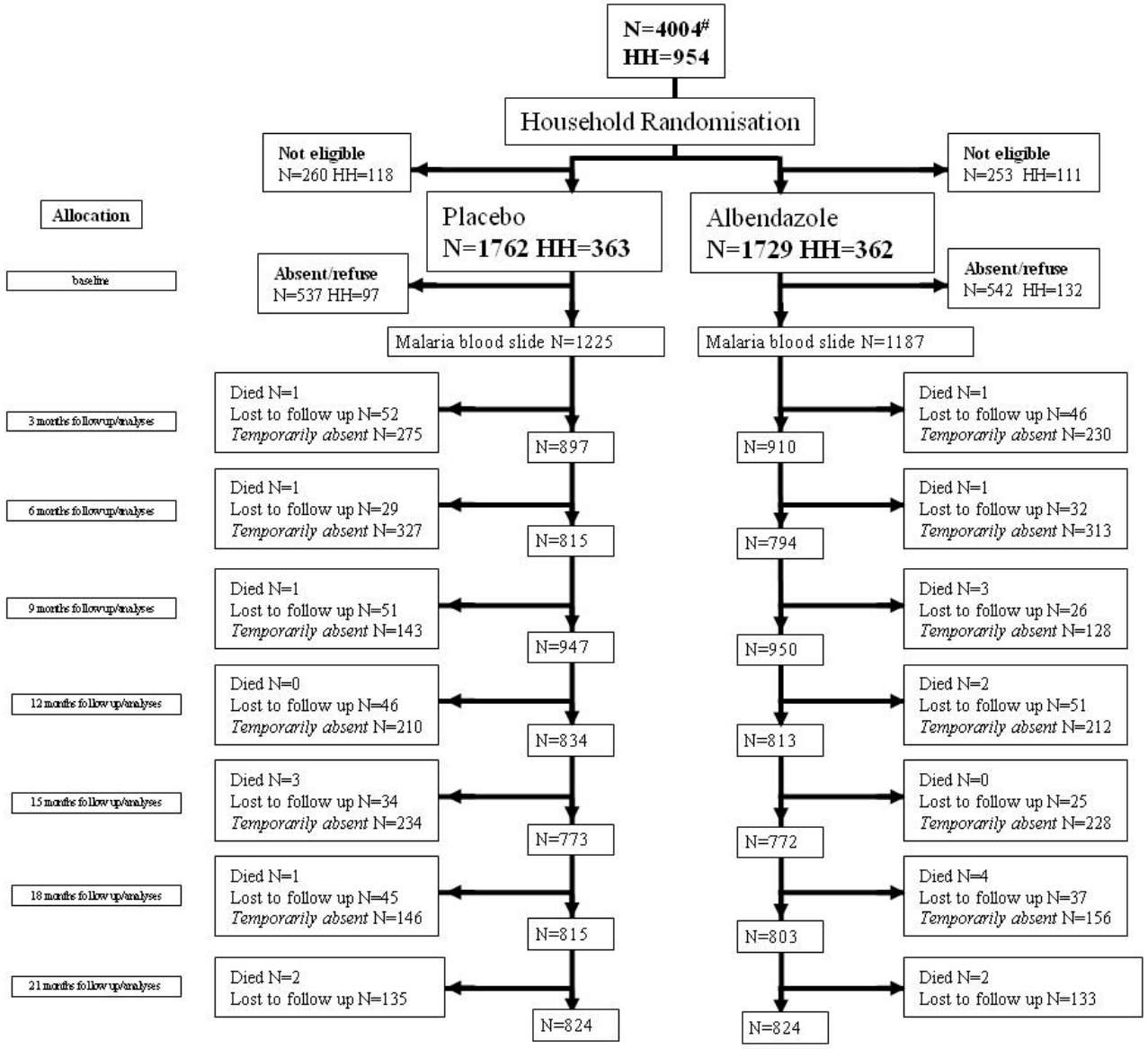

Figure S1B. Profile of trial with skin prick test (SPT) reactivity as outcome in children 5-15 years of age in both Nangapanda and Anaranda

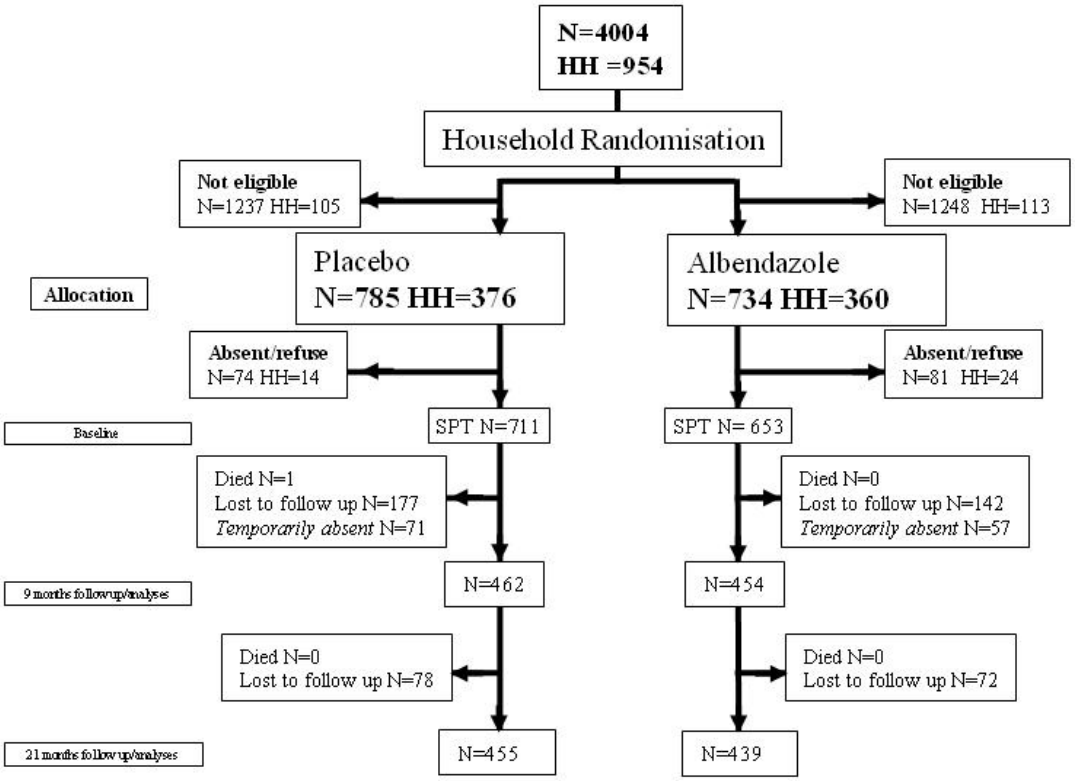

Figure S1C. Profile of trial with helminth infection as outcome in villages of Nangapanda and Anaranda

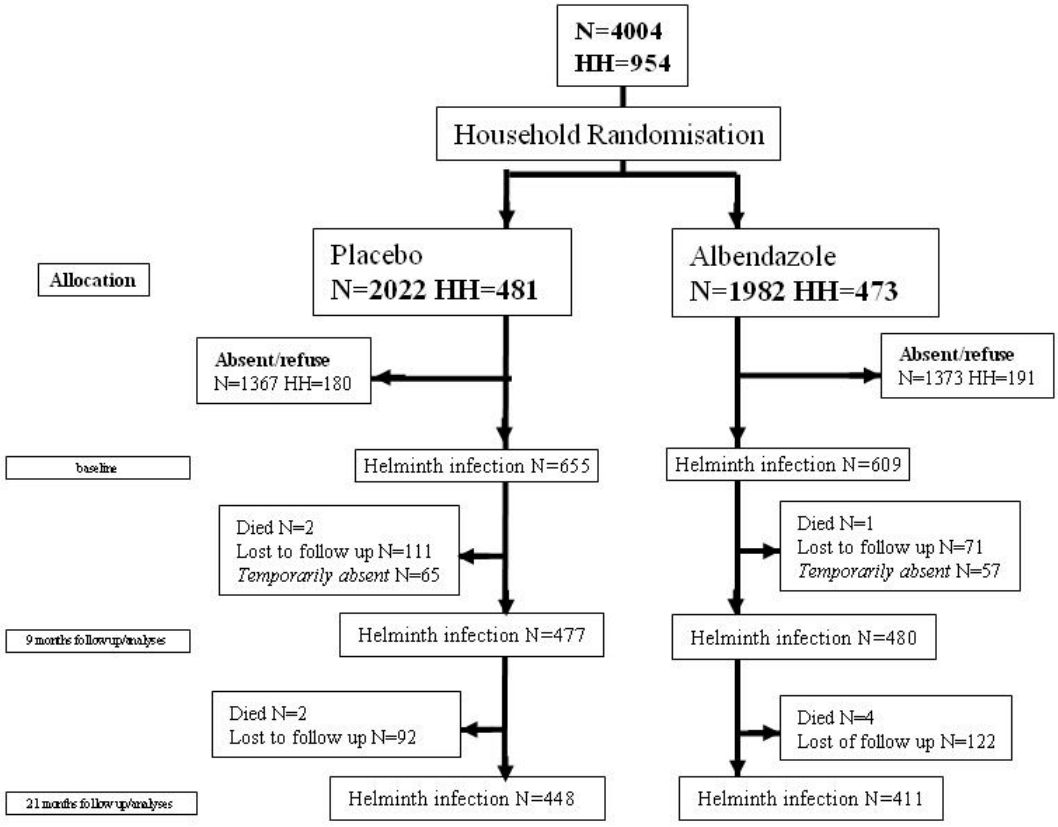

Table 1: CONSORT 2010 checklist of information to include when reporting a cluster randomised trial

Section/Topic	Item No	Standard Checklist item	Extension for cluster designs	Section
Title and abstract				
	1a	Identification as a randomised trial in the title	Identification as a cluster randomised trial in the title	Title
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)[1,2]	See table 2	Title and abstract
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	Rationale for using a cluster design	Introduction
	2b	Specific objectives or hypotheses	Whether objectives pertain to the the cluster level, the individual participant level or both	Introduction
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Definition of cluster and description of how the design features apply to the clusters	Methods
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		-
Participants	4a	Eligibility criteria for participants	Eligibility criteria for clusters	Methods
	4b	Settings and locations where the data were collected		Methods and in supplementary appendix S1
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Whether interventions pertain to the cluster level, the individual participant level or both	Methods and in supplementary appendix S1
Outcomes	6a	Completely defined pre-specified primary and	Whether outcome measures pertain to the cluster level, the	Methods

		secondary outcome measures, including how and when they were assessed	individual participant level or both	
	6b	Any changes to trial outcomes after the trial commenced, with reasons		-
Sample size	7a	How sample size was determined	Method of calculation, number of clusters(s) (and whether equal or unequal cluster sizes are assumed), cluster size, a coefficient of intracluster correlation (ICC or *k*), and an indication of its uncertainty	Methods and findings
	7b	When applicable, explanation of any interim analyses and stopping guidelines		Protocol S1 and in supplementary appendix S1
Randomisation:				
Sequence generation	8a	Method used to generate the random allocation sequence		Methods
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Details of stratification or matching if used	Methods
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Specification that allocation was based on clusters rather than individuals and whether allocation concealment (if any) was at the cluster level, the individual participant level or both	Methods and in supplementary appendix S1
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Replace by 10a, 10b and 10c	Methods and in supplementary appendix S1
	10a		Who generated the random allocation sequence, who enrolled clusters, and who assigned clusters to interventions	Methods and in supplementary appendix S1

	10b		Mechanism by which individual participants were included in clusters for the purposes of the trial (such as complete enumeration, random sampling)	Methods
	10c		From whom consent was sought (representatives of the cluster, or individual cluster members, or both), and whether consent was sought before or after randomisation	Methods
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		Methods and in supplementary appendix S1
	11b	If relevant, description of the similarity of interventions		-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	How clustering was taken into account	Methods and in supplementary appendix S1
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		Methods and in supplementary appendix S1
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	For each group, the numbers of clusters that were randomly assigned, received intended treatment, and were analysed for the primary outcome	Results and in supplementary appendix S1
	13b	For each group, losses and exclusions after randomisation, together with reasons	For each group, losses and exclusions for both clusters and individual cluster members	Results and in supplementary appendix S1

Recruitment	14a	Dates defining the periods of recruitment and follow-up		Methods
	14b	Why the trial ended or was stopped		Methods
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Baseline characteristics for the individual and cluster levels as applicable for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	For each group, number of clusters included in each analysis	Results
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Results at the individual or cluster level as applicable and a coefficient of intracluster correlation (ICC or k) for each primary outcome	Results
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		Results
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory		Results and in supplementary appendix S1
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[3])		Results and in supplementary appendix S1
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses		Discussion
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Generalisability to clusters and/or individual participants (as relevant)	Discussion

Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence		Discussion
Other information				
Registration	23	Registration number and name of trial registry		Abstract and methods
Protocol	24	Where the full trial protocol can be accessed, if available		Protocol S1 file
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders		Abstract

Table 2: Extension of CONSORT for abstracts[1,2] to reports of cluster randomised trials

Item	Standard Checklist item	Extension for cluster trials
Title	Identification of study as randomised	**Identification of study as cluster randomised (yes, p1 line 1-3)**
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	**p3, line 49.**
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	**Eligibility criteria for clusters (yes, p3 line 50-52)**
Interventions	Interventions intended for each group	**p3, line 51-52**
Objective	Specific objective or hypothesis	**Whether objective or hypothesis pertains to the cluster level, the individual participant level or both (yes, p3 line 47-48)**
Outcome	Clearly defined primary outcome for this report	**Whether the primary outcome pertains to the cluster level, the individual participant level or both (yes, p3 line 60-63)**
Randomization	How participants were allocated to interventions	**How clusters were allocated to interventions (yes, p3 line 50-51)**
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	**p3, line 52-53**
Results		
Numbers randomized	Number of participants randomized to each group	**Number of clusters randomized to each group (yes, p3 line 51-52)**
Recruitment	Trial status[1]	
Numbers analysed	Number of participants analysed in each group	**Number of clusters analysed in each group (yes, p3 line 51-52,58)**
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	**Results at the cluster or individual participant level as applicable for each primary outcome (yes, p3 line 59-64)**
Harms	Important adverse events or side effects	**p3, line 64**
Conclusions	General interpretation of the results	**p3 line 65-67**
Trial registration	Registration number and name of trial register	**p1 line 4, p3 line 68-69)**
Funding	Source of funding	**p3 line 68**

[1] Relevant to Conference Abstracts

REFERENCES

1 Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG, et al. CONSORT for reporting randomised trials in journal and conference abstracts. *Lancet* 2008, 371:281-283

2 Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG at al (2008) CONSORT for reporting randomized controlled trials in journal and conference abstracts: explanation and elaboration. *PLoS Med* 5(1): e20

3 Ioannidis JP, Evans SJ, Gotzsche PC, O'Neill RT, Altman DG, Schulz K, Moher D. Better reporting of harms in randomized trials: an extension of the CONSORT statement. *Ann Intern Med* 2004; 141(10):781-788.

Study program:
ImmunoSPIN program in Indonesia "Parasitic Infections and Inflammatory Diseases: The web of immune responses, host genetics and environmental exposure"

Trial protocol adjusted for manuscript:

The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: A household-based cluster-randomized, double-blind, placebo-controlled trial

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Background:

Major intervention programs have been launched, which use mass drug administration (MDA), often given annually and over protracted periods, to control STH. However, the evidence base is lacking on how effective MDA can be and whether it might result in the emergence of pathological conditions, as helminth infections are thought to have profound effects on the immune system.

Parasitic helminths have strong immunomodulatory activities affecting responses to third party antigens. In many parts of the developing world, malarial and helminth infections are co-endemic and many children are infected by both parasites. Investigation on the immunological associations between helminth infections and malarial parasites in co-endemic areas hold the key to answer the question whether helminths increase suppressory immune responses and increase susceptibility to malarial parasites on the one hand but protect from cerebral malaria on the other. Indonesia is a country in transition from one with high burden of infection and traditional lifestyle to one where infectious diseases will be controlled and modern lifestyle will take over bringing along an increase in inflammatory diseases such as allergies. So far most studies analyzing the effect of helminths on immune responses, malaria or allergies have been cross sectional. There are only two recent longitudinal study of the effect of anti helminths on allergy with a randomised placebo controlled design and none studying malaria outcomes; such studies are needed to establish the role that helminths play in suppressing immune responses and affecting malaria or allergies. For co-infections with malarial parasites, detrimental or protective effects of helminth infections will change policies regarding helminth control programs as would information on the mechanistic pathways such as immune responses that can provide valuable information for vaccines and drug design that aim to control infection or disease. Equally important is the results generated from studies involving the effect of helminths on inflammatory diseases such as allergies. These are diseases of populations with Western life style and as Indonesia is moving rapidly towards stronger economy and consequent increased urbanisation and adoption of Western life style, this program is getting prepared for what are expected to form serious future public health problems. The global initiative to control helminth infections worldwide is based on assumptions that these infections affect child health. Growth and cognition are thought to be adversely affected. However, a recent review challenges the consistency of such findings.

Altogether, well-controlled randomised trials are needed to provide us with robust data regarding the effects that helminth infections have on health and disease.

Key references

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Trial objectives and purpose:

- To assess the effect of albendazole on prevalence of malarial parasitemia and on fever and malarial-like symptoms.
- To assess the effect of albendazole on prevalence of SPT reactivity to allergens and symptoms of allergy.
- To assess the effect of albendazole on prevalence of soil-transmitted helminths.

Trial design

Household-based cluster-randomized, double-blind, placebo-controlled trial. Household clustering will be used in order to maximally interrupt the transmission of helminths.

Trial Endpoints:

Primary outcomes

- prevalence of malarial parasitemia
- prevalence skin prick test (SPT) reactivity to allergens
- prevalence of soil-transmitted helminths

Additional outcomes

- fever and additional malaria-like symptoms
- symptoms of allergy
- body mass index

Trial treatment:

Albendazole (400 mg) and identical pill as placebo were manufactured by PT Indofarma Pharmaceutical, Bandung, Indonesia. The treatment will be provided every three months for a period of 21 months after the start of treatment.

Randomization and blinding

The houses will be marked by Global Positioning System (GPS) system to allow maps to be generated using ARC GIS 9 software (ESRI, USA). Individual's geographical coordinates will be assigned as well as centre coordinates for households. Computer-aided block randomization by household, using Random Allocation Software will be used to assign treatment groups. The treatment will be coded as A or B 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 the study team located in Jakarta without the involvement of the field study investigators.

A number of nested studies will be conducted which will also use the Random Allocation software to apply to the treatment A and B arms and generate within each of these arms, sub samples for cellular immunological study. The treatment code will be de-blinded when all data needed for analyses are entered into the database.

Selection of study subjects

Inclusion criteria

Subjects of both sexes, aged 2 years and older, in good health, without any clinical condition requiring chronic medication and who have given informed consent (written or thumb print) will be recruited into the trial.

Exclusion criteria

Children younger than 2 years of age
Pregnant women
Subjects with clinical condition requiring chronic medication
No informed consent

Procedures for collection of samples

Methods and timing of assessments

Malaria parasite assessment by microscopy- at pre treatment and at 3, 6, 9, 12, 15, 18 and 21 months after the start of treatment.

Thick and thin blood smears will be prepared from finger prick blood. The slides will be stained with Giemsa and examined microscopically for all species of malaria parasites according to WHO SOP. The technicians reading the malaria slides will be certified at the US-Naval Medical Research Unit #2 (US-NAMRU2) in Jakarta. The outcome will be positive or negative to assess prevalence.

Malaria parasite assessment by Polymerase Chain Reaction (PCR)- at pre treatment and at 9 and 21 months after the start of treatment.

Blood kept frozen (at -20^{o} C) will be used for DNA extraction and analysis by PCR according to published protocols (Wiria et al., BMC Infect Dis. 2010 Mar 25;10:77 and Hamid et al., BMC Infect Dis. 2011 Apr 1;11:83). The test will be undertaken at LUMC.

Helminth parasite assessment by microscopy- at pre treatment and at 9 and 21 months after the start of treatment.

Stool containers will be distributed and collected by health workers and delivered to the field laboratory. Technicians trained and certified in Jakarta will perform the microscopic examination of the stool samples (Wiria et al., BMC Infect Dis. 2010 Mar 25;10:77 and Hamid et al., BMC Infect Dis. 2011 Apr 1;11:83).

Helminth parasite assessment by PCR- at pre treatment and at 9 and 21 months after the start of treatment.

Fresh stool samples will be kept frozen (at -20^{o} C) and used for DNA extraction and analysis by PCR to detect *Ascaris lumbricoides, Necator americanus and Ancylostoma duodenale* as well as *Strongyloides stercoralis*, according to published protocols (Wiria et al., BMC Infect Dis. 2010 Mar 25;10:77 and Hamid et al., BMC Infect Dis. 2011 Apr 1;11:83). The test will be undertaken at LUMC.

Fever and additional malaria-like symptom registration by questionnaires designed based on WHO recommended criteria at monthly visits by trained health workers. Fever will be measured by assessing oral temperature using digital thermometers.

The forms to record fever and additional malaria-like symptoms are appended (see appendix 1), will be used by trained health workers at monthly visits to households. Each health worker will be responsible for 20 houses. History of fever, malaria like-symptoms, health care visits and medications used are recorded within one month period. Individuals with fever and/or additional malaria-like symptoms within two days and at the time of visit are referred to the local primary health center (Puskesmas) for further assessment.

Skin prick testing- at baseline pre treatment and at 9 and 21 months post treatment assessed by one investigator. The skin prick testing (SPT) to allergens *Dermatophagoides pteronyssinus* and *farinae*, *Blatella germanica* is performed according to protocol published (Hamid et al., BMC Infect Dis. 2011 Apr 1;11:83). The study of allergic outcomes aims to look at school-age children and therefore this test will be restricted to school-age children in our study households.

ISAAC questionnaire administration- at baseline pre treatment and at 21 months after the start of treatment by one investigator

The ISAAC questionnaires developed specifically for study area during EU funded project GLOFAL (www.glofal.org) will be used along with visual material to assess the prevalence of reported allergic symptoms (Asthma, Rhinitis and Eczema).

The study of allergic outcomes aims to look at school-age children and therefore this test will be restricted to school-age children in our study households.

Anthropometry assessment-at baseline pre treatment and at 9 and 21 months after the start of treatment.

Height and weight will be measured using scales (standard SECA tools).

Blood sampling- at baseline pre treatment and at 9 and 21 months post treatment, 6 mls of peripheral blood collected into heparin tubes (BD vaccutainers) by experienced medical staff for use in immunological work. Plasma samples will be separated for measurement of antibody levels

Assessment of adverse events

Although albendazole is a well established safe drug, the study population will be informed to report any adverse reactions to field physicians and health workers. Adverse events spontaneously reported by the patient or observed by the investigators, will be monitored throughout the study. The reports will be sent to safety clinician responsible for adverse events reporting and assessed whether it is a trial related adverse event.

Data management

The database will be designed in MS Access and managed at LUMC but the latest database will be sent to the PI in Indonesia at weekly intervals as read only file. All data collected in the field at the monthly follow ups of fever and additional malaria-like symptoms will be entered by two trained data entry personnel after the forms are inspected and approved by study investigator. For each data series, procedure for double entry, quality control and coherence queries procedures will be implemented systematically in order to detect errors and/or missing data. After integration of all the data corrections on the whole data base, the data base will be locked and saved before it is transferred to the trial statistician. Each step of the process will be controlled by implementation of individual passwords and/or regular back-ups, in order to allow access to the data base and to ensure its integrity.

Confidentiality of individual data

Participant confidentiality will be strictly safeguarded by the participating investigators and their staff. This confidentiality is extended to cover testing of biological samples and possible future genetic tests in addition to the clinical information relating to participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party.

Statistics

Sample size calculation- The sample size calculation document was prepared by statisticians from LUMC and is provided in a document in appendix 2.

Analyses of Trial data- The LUMC will conduct the analyses of trial data for manuscripts approved by the publication committee. At a minimum, the trial manuscripts are expected to address the primary and secondary outcomes as well as the additional outcomes listed in the protocol.

Statistical methods- The appropriate statistical methods for longitudinal data and repeated measurements will be used. As the data are very complex, appropriate modeling will be needed in close collaboration with biostatisticians at LUMC to analyze the data.

Laboratory methods

The laboratory methods used have been published in 2 protocol papers. (Wiria et al., BMC Infect Dis. 2010 Mar 25;10:77 and Hamid et al., BMC Infect Dis. 2011 Apr 1;11:83).

Quality control

Several measures will be taken to assure a high level of quality control in all aspects of the study.
Questionnaires: health workers will attend regular training sessions to be trained and re trained by study investigators in order to keep to standard administration of questionnaires that record malaria-like symptoms.
All pipettes will be calibrated twice a year at LUMC and returned to the field laboratory.
The technicians conducting malaria and helminth assessment will be trained and re trained in Jakarta and in the field twice a year.
Double entry of selected fields will be undertaken, to assure that errors in data entry are minimized and the final dataset is as clean as possible.

Supplies

All supplies needed for the study not available in Jakarta will be shipped from the Netherlands to Jakarta and from Jakarta to Ende. The supplies will be the same (ie same company and product number) throughout the study.
The checklist of reagents will be drawn every 3 months and orders will be placed by fax or email (if internet connection works properly).
Storage room will be checked and lists will be updated at one monthly interval with study investigators being responsible for the updates.

Sample labeling

The labels will be of a quality that remains intact under all temperatures needed in the study. Before taking any sample, correct tubes and accessories shall be checked, and the tubes labeled accordingly. A label will contain ID number and a letter from alphabet to indicate the pre and post treatment follow up time points.

Sample storage

-Plasma samples will be stored at -20 ^{o}C
-Stool samples will be stored at room temperature (formalin preservation), -20 ^{o}C (fresh stool for PCR)
-Freezer temperature will be recorded regularly with thermometer.

Sample transport

Plasma, and stools will be transported to Jakarta on dry ice that will be flown into Flores via Bali or Jakarta.

Time frame

The study has a level of funding that was cut down considerably by the grant giving body. The aim was originally, when grant was written to conduct the study for a period of 3 years. However, financial constrains could lead to the earlier termination of the study. In addition, if the results from one and two year interim analysis indicate that it is not ethical to withhold albendazole to half of the study population, the trial will be ended.

Ethical issues and approval

The study will be submitted to the Committee of the Medical Research Ethics of the Faculty of Medicine of the University of Indonesia and to the LUMC Committee of Medical Ethics. Further, efforts will be made to ensure approval of the local village leaders and parents/guardians of the study children. To ensure ethical conduct of the trial, all the clinical investigators will follow courses on trial methods (ErasmusMC, Netherlands), courses on GCP and GLP organized by EU funded projects GLOFAL and COINFECT.

Informed Consent Process

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specified procedures or interventions are carried out. Information will be given in both oral and written form whenever possible. Independent witnesses will be required to attest that illiterate parents/guardians of potential participants have understood the contents of the informed consent.
It is expected that there will be extensive contact between the study team and the populations involved that should lead to the development of mutual trust and the establishment of an ongoing informed consent process attempting to address issues related to interventional studies in resource-limited settings. Many discussions with local community leaders, heads of families and citizens through group meetings, and more limited group interviews will be undertaken prior to trial start.
All relevant information on the possible risks such as discomfort and pain during blood sampling, the acceptance of health workers at monthly intervals they need to honor etc, will be explained. In all cases, the investigator will give the participants ample opportunity to inquire about the details of the trial and to ask any questions before dating and signing the consent forms. Informed consent approved by the ethics committee, will be signed or thumb-printed and dated by the participant's parent/guardian and by the person who conducted the informed consent discussion. Thumb printing will be used for illiterate persons only

Appendix 1

The forms to record fever and additional malaria-like symptoms

Date of Visit: ___ ___ . ___ ___ . ___ ___ ___ ___

Name of Subject: _____ Subject ID: ___ ___ ___ ___

Village: ___ Household: ___ ___ ___ Name of Cadre: _____

1. **Was subject ill since last visit ?** Yes No

   1a. **Symptoms:**

   Fever (including Chills and Sweating)
   Headache
   Fatigue (including Myalgia and Arthralgia)
   Vomiting
   other (specify): _____

   1b. **How long ago ?** _____ days ago / currently ill?

   1c. **Duration of symptoms** : _____ days

   1d. **Temperature (if fever or history of fever):** _____ °C

2. **Did subject take treatment for illness ?** Yes No

   2a. **Who gave treatment ?** self-medication puskesmas doctor other

   2b. **Name of drug (incl. traditional herbs)**: _____

3. **Did subject visit health care center since last visit ?** Yes No

   3a. **Which ?** puskesmas doctor hospital other

   3b. **Date of visit:** ___ ___ . ___ ___ . ___ ___ ___ ___

   3c. **hospitalized?** Yes No

4. **Notes:**

Appendix 2

Sample Size Calculation for ImmunoSPIN project

Calculation are based on data from pilot study and from literature.

For all calculations power is set at 0.90 and significance level at 0.05

SKIN PRICK TEST

The 4th column gives the number of people needed in either treatment arm to find a post treatment SPT prevalence as predicted in column 2. In column 5 the number of individuals in either treatment arm needed, presuming that 20% of the people may be lost to follow up (LTFU). The last column gives the total number of enrolments before randomization under this condition.

Prevalence (P_0) -control group-	Predicted prevalence (P_1) - treatment group-	RR	N = treated	If 20% LTFU	Total enrolments (before randomization)
HDM 15%	17.5%	1.2	4567	5720	11440
	20%	1.3	1212	1515	3030
	22.5%	**1.5**	**567**	**709**	**1418**
	25%	**1.7**	**334**	**418**	**835**
	27.5%	1.8	223	279	558
	30%	2	161	242	484
Cockr. 35%	37.5%	1.07	7771	9714	19428
	40%	1.14	1969	2461	4922
	42.5%	1.21	885	1106	2212
	45%	**1.29**	**502**	**628**	**1256**
	47.5%	**1.36**	**324**	**405**	**810**
	50%	1.43	226	283	566

MALARIAL PARASITEMIA

Prevalence (P_0) -control group-	Predicted prevalence (P_1) - treatment group-	RR	N = treated	If 20% LTFU, Treatm. failure	Total enrolments (before randomization)
20%	17.5%	0.88	5122	6403	12805
	15%	**0.75**	**1212**	**1515**	**3030**
	14%	**0.70**	**822**	**1028**	**2056**
	12.5%	0.63	507	634	1268
	10%	0.5	266	333	666
10%	7.5%	0.75	2684	3355	6710

	6.75%	0.67	1525	1906	3812
	6%	**0.60**	**965**	**1206**	**2412**
	12.5%	1.25	3356	4206	8412
	13.75%	1.38	1562	1953	3906
	15%	**1.5**	**918**	**1148**	**2295**

Key reference

van den Biggelaar AH, Borrmann S, Kremsner P, Yazdanbakhsh M. Immune responses induced by repeated treatment do not result in protective immunity to Schistosoma haematobium: interleukin (IL)-5 and IL-10 responses. J Infect Dis. 2002;186:1474-82.

van den Biggelaar AH, Rodrigues LC, van RR, van der Zee JS, Hoeksma-Kruize YC, Souverijn JH et al. Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. J Infect Dis 2004;189:892-900.