

## Confirmation of the Protective Effect of *Ascaris lumbricoides* on *Plasmodium falciparum* Infection: Results of a Randomized Trial in Madagascar

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**Abstract.** A controlled randomized trial of anti-helminthic treatment was undertaken in 1996–1997 in a rural area of Madagascar where populations were simultaneously infected with *Ascaris lumbricoides*, *Plasmodium falciparum*, and *Schistosoma mansoni*. Levamisole was administered bimonthly to 107 subjects, whereas 105 were controls. Levamisole was highly effective in reducing *Ascaris* egg loads in the treated group ( $P < 10^{-3}$  at all visits), whereas it had no effect on schistosomiasis. Subjects 5–14 years of age, treated with levamisole, had a significant increase of their *P. falciparum* densities compared with controls ( $P = 0.003$ ). There was no effect of the treatment on children 6 months to 4 years of age, nor on adults > 15 years of age. This study confirms the results of a randomized trial, which showed a negative interaction in those > 5 years of age between *Ascaris* and malaria parasite density in another Malagasy population, submitted to a higher malaria transmission.

### INTRODUCTION

Infection with helminths is strongly suspected to play a role in malaria in endemic areas, by acting either on the infection (incidence, parasite load) or on the risk of clinical disease. Published studies may seem contradictory, because they show either a protective or a worsening effect. Recently in endemic areas, several studies showed antagonistic effects between malaria and trematodes,<sup>1,2</sup> whereas others showed a synergistic interaction between malaria and *Ascaris*.<sup>3</sup> In fact, such diverging results apply to different helminth species (mainly *Ascaris* and *Schistosoma* sp.), different transmission settings, and different outcomes (clinical or parasitologic patterns, on particular population subgroups).<sup>4</sup> In addition, interpretations can be biased by the lack of adjustment on confounding factors such as age or location, and thus differential exposure to the parasites, in most observational studies.<sup>5</sup>

A more radical way to eliminate the influence of confounders in field studies is to study the influence of a pathogen on another by a randomized trial, aiming to suppress one of the two parasites. We recently published the results of a randomized trial of anthelmintic treatment in a population simultaneously infected with *Ascaris lumbricoides* and *Plasmodium falciparum* in Madagascar, showing an increase in *P. falciparum* densities in children > 5 years of age, treated with levamisole, and thus an antagonistic effect between *Ascaris* and malaria.<sup>6</sup>

At the same time, we performed a similar trial on a different population living at a higher altitude, also affected by malaria and ascariidiosis, but also situated in a *Schistosoma mansoni* transmission area.

We report here the results of this trial.

### MATERIALS AND METHODS

**Study area.** The general setting of the study has been described in detail elsewhere.<sup>6</sup> Briefly, this study was conducted in the highland village of Fenoarivo (altitude, 1,250 m), located 300 km southwest of Antananarivo, in a zone targeted

by a routine DDT house-spraying program (Op ration de Pulv risation Intra-Domiciliaire de DDT [OPID]) from 1993 to 1998. It is a hilly area, where rice is cultivated on terraces. It enjoys a tropical mountain climate, with a warm and humid season from November to March and a cool and dry season from April to October. *A. lumbricoides*, *Plasmodium* sp., and *S. mansoni* are the main parasites affecting the human population. Malaria is hypoendemic and transmission occurs mainly from January to June,<sup>7</sup> whereas helminths are transmitted all year round.<sup>8</sup>

**Study population and protocol.** Similarly to the first trial in the village of Ambohimena, families were randomly allocated to a treatment or control group. The same rules of inclusion/exclusion were applied (namely, inclusion after oral consent, exclusion of infants < 6 months of age). Treatment assignment was not blinded, and inclusion of new families was stopped after the third visit.

The same variables were studied by questionnaires given at inclusion: age, sex, notion of ongoing pregnancy or clinical symptoms such as diarrhea or fever in the preceding days, intake of drugs, or recent migration outside of the study area.

Village residents were visited by investigators every 2 months during the 18 months of the study (June 1996 to October 1997). They were clinically examined, stool and blood samples were taken for malaria parasites, and there was a helminth search. Updating of questionnaires was also done on the same occasion. In addition, subjects belonging to the treated group were administered at each visit an anti-helminthic single-dose oral treatment of levamisole (3 mg/kg in children, 150 mg in adults) under control by the investigators. A multivitamin treatment (0.5–3 tablets every 2 months, each tablet containing 2,500 IU vitamin A, 1.0 mg thiamine, 0.5 mg riboflavin, 7.5 mg nicotinamide, and 300 IU vitamin D<sub>3</sub>) was given to the subjects belonging to the control group. All villagers infected with malaria parasites were treated with chloroquine sulfate (25 mg/kg over 3 days) according to the official treatment policy in Madagascar. At the end of the study, all the population (both treated and control groups) received levamisole.

The protocol was given ethical approval by the Madagascar Ministry of Health (Direction de la Lutte contre les Maladies Transmissibles [DLMT]).

**Biological methods.** Stool samples were processed by the merthiolate iodine formalin (MIF) concentration method on

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a calibrated amount of stool, which allows staining, concentration, and count of helminth eggs (*A. lumbricoides*, as well as *S. mansoni* and *N. americanus*).

At each visit, fingerprick thick and thin blood smears were made on all subjects. Smears were stained with Giemsa, and 100 oil-immersion microscopic fields were examined for malaria parasites. Parasites and white blood cells were enumerated, and parasite density was calculated according to an assumed average of 8,000 leukocytes/mm<sup>3</sup>.

**Statistical analysis.** We first performed a univariate analysis to compare the two groups on entering the study on the basis of general characteristics and prevalences and densities of the two main parasites (*P. falciparum* and *A. lumbricoides*). The  $\chi^2$  test was used to test differences in proportions, and Student *t* test or non-parametric Wilcoxon test was used to test differences in means.

We examined malaria parasite density in relation to the treatment group. Only *P. falciparum* densities, which represented 94% percent of all diagnosed parasites, were analyzed. *P. malariae* accounted for 5% percent and *P. vivax/P. ovale* for 1% percent of single malarial infections. *P. falciparum* densities and helminth egg loads were log-transformed [ $\text{Log}(\text{DP} + 1)$ ] to reduce distribution asymmetry, which is usual in parasite infection data.

Because the study design was a randomized trial, we verified that subjects allocated to repeated antihelminthic treatment and controls were similar for a number of variables (Table 1) that we did not take into account in further analyses.

As in the preceding trial, we analyzed repeated measures of parasite densities (1–9 per individual, mean 7.91) on subjects belonging to 29 distinct families. To deal with this nested structure, we performed an ANOVA on parasite density, where the nested factors family and subject were considered as random.

According to the association between age and immunity to

malaria, three age groups (6 months to 4 years, 5–14 years, and  $\geq 15$  years) were defined, in which we tested the association between *P. falciparum* density and treatment group by a stratified analysis.

Our analysis was performed using the following model:

$$Y_{ijk} = \underbrace{\beta_0 + \beta_1 X_{ij}}_{\text{fixed effects}} + \underbrace{a_i + b_{ji} + e_{ijk}}_{\text{random effects}}$$

with  $a_i \sim N(0, \sigma_a^2)$  (between-family variation);  $b_{ji} \sim N(0, \sigma_b^2)$  (between-subject within-family variation);  $e_{ijk} \sim N(0, \sigma_e^2)$  (within-subject within family variation);  $Y_{ijk}$  is the  $k$ th  $\log(P. falciparum$  density + 1) for subject  $j$  from family  $i$ , and  $X_{ij}$  is a dichotomous variable that equals 0 for subject  $j$  belonging to an untreated family  $i$  and equals 1 for subject  $j$  belonging to family  $i$  that received levamisole.

This model has two fixed parameters that are, for  $\beta_0$ , the overall mean of *P. falciparum* density (log-transformed), and for  $\beta_1$ , the effect of treatment of *A. lumbricoides* and three random effects variances that need to be estimated. The nested random effects imply the following covariance structure for  $Y$ s measured on the same subject from the same family:

$$\begin{pmatrix} \sigma_a^2 + \sigma_b^2 + \sigma_e^2 & \sigma_a^2 + \sigma_b^2 & \sigma_a^2 + \sigma_b^2 \\ \sigma_a^2 + \sigma_b^2 & \sigma_a^2 + \sigma_b^2 + \sigma_e^2 & \sigma_a^2 + \sigma_b^2 \\ \sigma_a^2 + \sigma_b^2 & \sigma_a^2 + \sigma_b^2 & \sigma_a^2 + \sigma_b^2 + \sigma_e^2 \end{pmatrix}$$

The covariance for  $Y$ s measured on different subjects is  $\sigma_a^2$ .

A more complex model including a continuous variable representing the average egg load of *S. mansoni* on the whole duration of the follow-up was also estimated to test the effect of this parasite on malaria parasite densities.

We estimated the mixed model parameters with PROC MIXED SAS procedure (SAS Institute, Cary, NC).

## RESULTS

**Study population.** Two hundred twelve subjects belonging to 29 families were enrolled in the study. After a random sampling of families, 107 subjects were assigned to the treated group, whereas 105 were controls. The two groups were compared on the basis of age, sex, number, and size of families and parasite infections (*Plasmodium falciparum*, *Ascaris lumbricoides*, *Schistosoma mansoni*, and *Necator americanus*). Table 1 shows that, on entering the study, no significant difference between the two groups was recorded for any of these variables. For *Necator americanus* infection, only one subject was infected in the control group and none were infected in the treated group (data not shown).

During follow-up, we lost contact with seven subjects after the first visit (six in the treated group and one in the control group), and we excluded from the analysis seven more subjects (five treated and two controls), who had been followed-up for a short duration (< 6 months).

In the control group, at all visits except one (in the month of October 1996, one single suspected malaria attack), no subject showed an association of fever and parasitemia, with an indication for the administration of oral chloroquine. In the levamisole-treated group, there were four suspected clinical malaria occurrences (two in June 1996, one in February, and one in April 1997) who received chloroquine.

TABLE 1

Characteristics of treated and control subjects at inclusion (before anti-helminthic treatment, Fenoarivo village, 1997)

	Treated (N = 107)	Controls (N = 105)	P
Age (years)			
0–4	18	20	0.79*
5–14	36	31	
15 and over	53	54	
Sex			
Male	54	47	0.41*
Female	53	58	
Family			
Number	16	13	—
Mean no. of subjects/family	6.7	8.1	0.33†
<i>Ascaris lumbricoides</i>			
No. infected subjects	60	58	0.82*
Prevalence rate	58.3%	59.8%	
Mean egg load	2.34	2.27	0.81‡
<i>Plasmodium falciparum</i>			
No. infected subjects	7	6	0.83*
Prevalence rate	6.7%	6.0%	
Mean parasite load	0.30	0.34	0.53†
<i>Schistosoma mansoni</i>			
No. infected subjects	47	41	0.63*
Prevalence rate	45.6%	42.3%	
Mean egg load	1.02	0.88	0.50‡

\*  $\chi^2$  test.

† Wilcoxon test.

‡ Student *t* test.

**Effect of levamisole treatment on *Ascaris* and other helminths.** At their inclusion in the study, all subjects from the treated group had received a therapeutic dose of levamisole, which was repeated on each further visit. By the end of the study, each of them had received an average of 7.5 doses.

As shown in Figure 1A, *Ascaris* egg loads immediately collapsed after the first visit in the treated group, whereas they persisted at a high level throughout the follow-up in the control group (all  $P < 0.001$  for each visit between the two groups). Prevalence rates followed a similar pattern (data not shown). However, it was noticeable that both prevalence and egg load progressively increased after 1 year of follow-up, which could be caused by a phenomenon of resistance to the administered treatment.

With regard to *S. mansoni*, despite a high infection rate in the whole population, there was no difference between groups for egg loads or prevalence at any of the nine visits (all  $P > 0.05$ ; see Figure 1B for egg load distribution).

**Effect of anti-helminthic treatment on plasmodial infection.** Mean *P. falciparum* densities in treatment groups are shown in Figure 2 (Figure 2A, all subjects; Figure 2B, 6 months to 4 years; Figure 2C, 5–14 years; Figure 2D,  $\geq 15$  years). There were few variations from 1 month to the other, because transmission stays rather low in the central highlands, and the span of seasonal parasitic fluctuation is quite narrow. Probably for the same reasons, there was no clear tendency to a decrease in the mean parasite density in the oldest age group. Because malaria infections are not as frequent in this population as in the less elevated areas, there is little chance for an individual to build up a protective immune response. Treatment and control groups varied in similar ways, with a tendency to higher densities in the treated group, mainly present in the age class 5–14 years.

Mixed model analysis (Table 2) confirmed that subjects treated with levamisole had a significant increase in their *P. falciparum* densities compared with controls ( $P = 0.007$ ). The

potential effect of treatment on parasite density was almost exclusively present in the age group 5–14 years ( $P = 0.003$ ). There was no effect of levamisole treatment on children 6 months to 4 years, nor on adults  $\geq 15$  years of age.

The model including *S. mansoni* did not show any effect of this parasite on the whole population, nor on the age group 5–14 years ( $P = 0.19$  and  $P = 0.29$ , respectively).

## DISCUSSION

In this study, we present the results of a controlled clinical trial of anti-helminthic treatment, which confirms the existence of a significant negative interaction between *Ascaris* infection and malaria parasite density in children 5–14 years of age. We performed a first randomized trial (the first ever done in a malaria-helminth co-infected population) in another Malagasy area,<sup>6</sup> which had shown a suppressive effect of *Ascaris lumbricoides* on malaria parasites in children  $> 5$  years of age.

The first trial was undertaken in a village on the western fringe of the central highlands of Madagascar where malaria is mesoendemic with a low transmission rate (three to nine infective bites per man per year),<sup>7</sup> whereas this study was performed in a more elevated area, targeted by routine yearly DDT house-sprays from 1993 to 1998. In this latter setting, malaria is hypoendemic and unstable, and transmission is much lower than in the former (less than one infective bite per man per year), with marked within- and between-year variations.<sup>9</sup> As these differences in malaria transmission may explain, at least in part, that the concerned age groups are not exactly the same, it is, however, striking that in both studies, a negative *Plasmodium*–*Ascaris* interaction was significant in children 5–15 years of age. In addition, in this study, four clinical malaria accesses (association of fever and parasitemia) were suspected in the levamisole-treated group versus one only in the control group.

It is also interesting that nearly 30 years ago, another anti-helminthic intervention in the Comoros, although not randomized and including a limited number of subjects, suggested that severe infection with *Ascaris lumbricoides* in 2- to 14-year-old children was associated with the suppression of malaria symptoms and showed that the anti-helminthic treatment was followed by a recrudescence of malaria.<sup>10</sup>

In general, the study of parasite co-infections has led to conflicting results. In concurrent experimental infection of mammalian hosts, the main available studies of *Plasmodium*/helminths co-infections showed suppression of different species of *Plasmodium* by *Schistosoma mansoni*, *Strongyloides ratti*, and *Trichinella spiralis*.<sup>11</sup>

Concerning *Ascaris* infections in human hosts, several observational studies have been made. A protective effect on particular clinical forms of human malaria, such as cerebral malaria or acute renal failure, has been suggested by Nacher and others,<sup>12,13</sup> whereas another recent study led to opposite results, showing a facilitating effect of *Ascaris* on severe malaria.<sup>3</sup>

Numerous observations of *Schistosoma*–*Plasmodium* co-infections have also been published. On these two parasites, the importance of helminth infection intensity has been stressed by several authors. For example, two studies held in Senegal and Mali showed a negative interaction between *P. falciparum* and *Schistosoma haematobium* light infections,<sup>1,2</sup> whereas a third study found a synergistic effect between *P.*

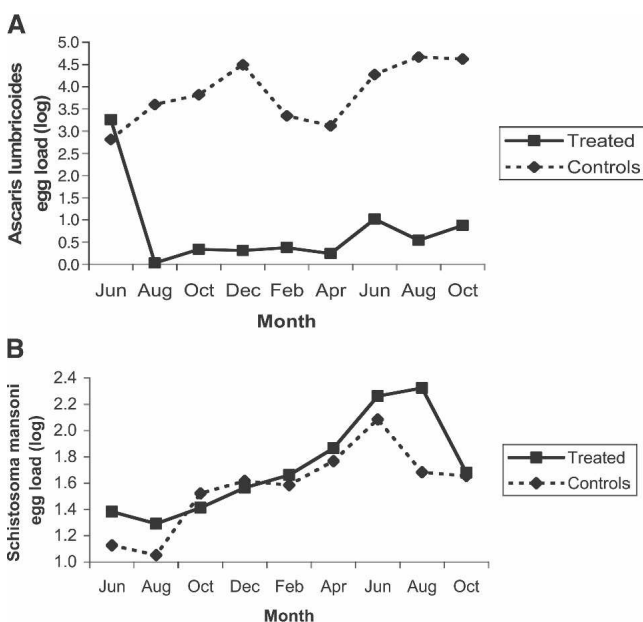


FIGURE 1. Mean egg loads (*A. lumbricoides* [A] and *S. mansoni* [B], log-transformed) in treated and control groups during the follow-up, Fenoarivo, 1997.

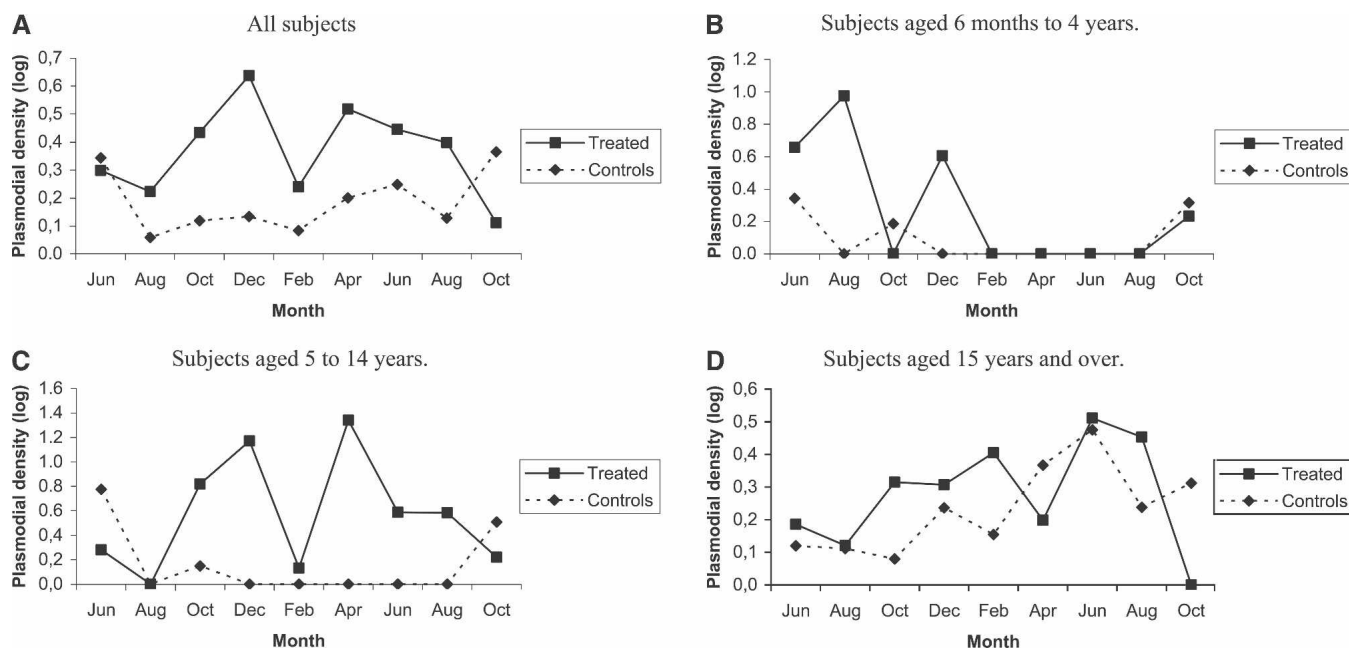


FIGURE 2. Mean parasite densities (*P. falciparum*, log-transformed) in treated and control groups by age group during the follow-up, Fenoarivo, 1997. **A**, All subjects. **B**, Subjects 6 months to 4 years of age. **C**, Subjects 5–14 years of age. **D**, Subjects > 15 years of age.

*falciparum* and high *S. mansoni* egg loads only.<sup>14</sup> Lyke and others<sup>2</sup> pointed out the age-dependent distribution of the protection conferred by schistosomiasis against falciparum infections. It can be explained by the fact that helminth infections are generally more prevalent and intense in school-aged children.<sup>15</sup> Malaria infections may occur at the same age, especially in areas where a low transmission delays the onset of a specific immunity. Because of the high exposure to both parasites simultaneously, it is thus logical that the interaction appears prominently in school-aged children.<sup>4</sup> In a similar way, this study showed a maximal protection of *Ascaris* against *P. falciparum* in 5- to 15-year-old children, who had the highest helminth egg loads and prevalences (71% versus 49% and 55% for children  $\leq$  5 years and adults, respectively).

As emphasized by Mwangi and others,<sup>4</sup> it is difficult to make valid comparisons between the findings from all these studies, because there are differences in terms of design studies, measurement of outcomes, parasites species, age groups, study populations, and controls. Observational studies are subject to biases, and there is a need to take appropriate account of all potentially confounding factors in the data analysis, usually unknown to the study designer. For example, observing an association between two species of parasites and their associated morbidity in the absence of control for location of residence may only be caused by a differential exposure to the parasites in a particular group of individuals and cannot be interpreted as evidence of biological interaction.<sup>5</sup>

To control location in co-infection, either stratification or matching can be used,<sup>1,2</sup> but well-designed, randomized clinical trials provide the most appropriate way to study the association between malaria and helminths.<sup>4</sup> To our knowledge, only two studies<sup>6</sup> including this one on parasitic co-infections have used a randomized trial design. It guarantees that no major bias could interfere with the main results, which were similar in both trials. In addition, we used for the statistical analysis a multivariate model to control potential confounding factors, and its hierarchical structure allowed to deal with all available information, suppressing biases on the estimators' variances.<sup>16</sup>

It is noteworthy that, in our intervention study, the introduction in the model of a *S. mansoni* variable allowed us to rule out the role of this additional parasite. To selectively suppress *Ascaris*, we decided to use levamisole at the total single dose of 3mg/kg instead of other anti-helminthics such as mebendazole or albendazole which have important deleterious effects on almost all species of intestinal helminths.<sup>17</sup> To our knowledge, levamisole has no efficacy on the treatment of trematodes. There is then no particular reason for a selective interaction of *S. mansoni* with either of the groups. This was further verified in the analysis of the data, because there was no difference in either prevalence or egg loads between treatment groups for *S. mansoni*, at any of the visits after inclusion.

In our mind, the fact that levamisole has also been used as

TABLE 2

Relation between *P. falciparum* density (log-transformed) and treatment of *A. lumbricoides* in different age groups. Fenoarivo, 1997

	Effect of treatment [95% CI]	<i>P</i> *	Intercept† [95% CI]	<i>P</i> †
Whole population ( <i>N</i> = 198)	0.25 [0.07–0.42]	0.007	0.17 [0.04–0.29]	0.008
0–4 ( <i>N</i> = 37)	0.15 [–0.05 to 0.35]	0.14	0.07 [–0.07 to 0.20]	0.34
5–14 ( <i>N</i> = 56)	0.58 [0.20–0.95]	0.003	0.08 [–0.19 to 0.35]	0.54
15 and over ( <i>N</i> = 105)	0.08 [–0.18 to 0.33]	0.55	0.24 [0.07–0.42]	0.007

\* One way nested repeated-measures ANOVA (see text).

† Intercept = baseline parasite density within the age group. Test of intercept to 0.

an immune-response regulator does not harm the significance of our results. Indeed, levamisole, as others anti-helminthics like thioimidazoles or ivermectin, is known for its immunomodulatory action.<sup>18</sup> It has a dose-dependent effect at higher doses than were used in our study. It consists in an overall stimulation of the cellular response that should increase resistance to parasite infections and reduce malaria parasite densities,<sup>19</sup> instead of increasing them as shown in this study.

Interestingly, we observed a slight increase in prevalences and egg loads of *Ascaris* in the untreated group but also in the "levamisole" group at the end of the survey (Figure 1A). Although we cannot exclude that there may exist a seasonal variation in the transmission pattern of *Ascaris* in this region, the most probable explanation is that our treatment schedule induced a resistance to levamisole in *A. lumbricoides* populations. This phenomenon has never been described in human communities but has been often reported in animals submitted to intensive and prolonged use of anthelmintics in systematic mass treatments.<sup>20</sup>

In conclusion, two successive clinical trials of anti-helminthic treatments in different populations gave similar results on the interaction between *Ascaris* and *P. falciparum*. It seems indeed that worms have a protective, rather than worsening, effect on malaria in terms of parasite load, at least for these two parasites, and contrary to Druilhe and others,<sup>21</sup> we definitely think that treating intestinal worms could not be a serious way to roll back malaria.

Such considerations have practical implications on the strategy of anti-malarial interventions. In particular, new malaria vaccine candidates have been tested in the field with promising results, such as RTS,S/AS02A.<sup>22</sup> In malaria-endemic areas, the majority of patients are already infected by helminths. As stressed by Nacher,<sup>23</sup> worms can act as an anti-adjuvant to vaccines, mainly by inducing a bias toward Th2 cytokines and by depressing interferon (IFN)- $\gamma$  release, with a risk of underestimation of the true effect of vaccines and of reduction of the power of vaccine trials in such co-infected populations. Furthermore, the protection of children against malaria may worsen the egg load of helminths in the vaccinated group. For all these reasons, it seems strongly advisable that, before any preventive or curative intervention against malaria in children exposed to intestinal worms, a radical treatment against helminths should be applied.

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