Interactions between Gastrointestinal Nematodes and Malaria in a Cohort of Children in an Amazonian Village

Aurelia Stefani, Marie Cheuret, Duc N'guyen, Stéphane Simon, Paul Brousse, Bernard Carme, Mathieu Nacher

Interactions between gastro intestinal nematodes and malaria in a cohort of children in an Amazonian village.

Aurélia Stefani[1],[2], Marie Cheuret[3], Duc N'Guyen[3], Stéphane Simon[1], Paul Brousse[4], Bernard Carme[1],[2],[5], Mathieu Nacher[1],[2},[5].

1. EPaT team, (EA 3593), UFR de Médecine – Université des Antilles et de la Guyane, Cayenne, French Guiana.

2. STRonGer Programme, Institut Pasteur de la Guyane, Cayenne, French Guiana.

3. Laboratoire de Parasitologie-Mycologie, Cayenne General Hospital, Cayenne, French Guiana.

4. Département des Centres de Santé, Cayenne General Hospital, Cayenne, French Guiana.

5. Centre d'Investigation Clinique (CIC INSERM 1424), Cayenne General Hospital, Cayenne, French Guiana INSERM.

Corresponding Author: Pr Mathieu Nacher, CIC INSERM 1424, Centre Hospitalier de Cayenne, Avenue des Flamboyants, 97300, Cayenne, French Guiana

Summary

Introduction. Most studies on nematode malaria interactions were conducted outside of the Americas. The objective of the present study was thus to study the relation between malaria and nematodes in a cohort of children in an Amazonian village.

Methods. Odds ratios for intestinal nematode infections as an explanatory variable to malaria-resistant vs. malaria-sensitive were computed.

Results. *Ascaris lumbricoides* was significantly more frequent in the “resistant” malaria group than in the “sensitive” one.

Conclusions. Despite its low statistical power, the present results find that **Ascaris** was associated with less malaria, as observed by a number of studies.

Keywords: *Plasmodium falciparum*, *Plasmodium vivax*, relapses, GI nematodes, *Ascaris lumbricoides*, French Guiana.

Introduction

Gastrointestinal nematode infections and malaria have a broadly overlapping distribution. A number of studies from different continents have shown complex interactions between different GI nematodes and malaria.

Observational studies in Thailand showed that *Ascaris lumbricoides* was associated with a dose-dependent association with protection from cerebral malaria and acute renal failure [1, 2]. Other studies have shown that *Ascaris* was associated with lower incidence or prevalence of malaria [3, 4]. In contrast, it was observed notably in Africa and Madagascar, that hookworm was associated with a greater incidence of malaria [5-9].

These research questions have received relatively little attention given the omnipresence of coinfections in tropical regions. The difficulty of this question lies in the different dynamics of transmission between malaria and different helminthes with different immunomodulatory properties or hematologic consequences, which complicates the analysis of results. In addition, most results come from observational studies which are prone to biases and confounding (nutritional status, socioeconomic level, anemia, background immunity, self-treatment, etc.).

In French Guiana, studies performed in 2000 - 2005 (unpublished data) have shown the persistence of a high prevalence of gastrointestinal nematode infections among communities living in the interior of French Guiana, particularly among Amerindian children living along the middle and upper Oyapock River (Camopi, Trois-Sauts). The most frequent parasite species were *Ascaris lumbricoides*, *Strongyloides stercoralis*, and *Necator americanus*. These studies have shown a prevalence exceeding 20% for these three nematodes.

In parallel, the incidence of malaria was high among children (0-7 years) in Camopi over the 2001-2009 period with a mean of 238, 514 and 21‰ person-years for *P. falciparum*, *P. vivax and mixed infections* (microscopic diagnosis), respectively. Finally, a univariate Cox regression analysis showed that there was a link between anthelminthic treatment and malaria in these children [10].

The trends suggest that while malaria seems less symptomatic among *Ascaris*-infected persons than those without *Ascaris*, it seems that incidence is higher among patients with hookworm than those without hookworm. This may result from immune modulation in *Ascaris*-infected patients and hematologic factors in hookworm-infected patients. Although discernible trends seem to emerge, there are conflicting results, which are often due to methodological differences. It is impossible to demonstrate causal relations in observational studies. However, converging elements may point to causal relations between two variables. To this end repeating the study in different contexts is important to determine if a finding is robust. Most studies on GI-nematode malaria interactions were conducted outside of the Americas except two studies in Colombia and Brazil [4, 7]. The objective of the present study was thus to determine whether there were any relation between malaria and GI nematodes in the context of a cohort of children under seven years of age living in a small Amazonian village in French Guiana.

Methods

The village of Camopi, located in the Oyapock malaria endemic area, consists of a central village and 28 hamlets localized within 15 km2 along the Oyapock and the Camopi Rivers. The village is isolated from the coast and separated from Brazil by the Oyapock River, which represents the border. The 1200 inhabitants of Camopi are mainly Wayampi and Teko Amerindians from the linguistic family of the tupi-guarani. They respectively live on the banks of the Oyapock and the Camopi Rivers. The population is young with an average age of 18 years.

The patients from the Camopi cohort described elsewhere[10, 11] were reviewed and classified in two groups, one resistant and one sensitive according to the number of malaria episodes. *Plasmodium vivax* relapses were defined as infections occurring within 90 days of a first *P. vivax* episode [12.]

Two groups of children were identified regarding their past history of malaria infection in the period 2001-2009: one “sensitive” group had ≥7 malaria episodes; and one "resistant" group only had ≤1 within 3 years or ≤2 within 6 years.

Subsequently to this group definition, three field missions were conducted to collect stool samples in order to test the hypothesis that malaria-resistant patients had a significantly different prevalence of GI nematodes than the "malaria-sensitive" group.

Overall 91 stool samples were collected. All past malaria history, nematodes infection and anthelmintic treatments were recorded for each child included in the study.

Laboratory diagnosis of three helminth infections was performed by multiplex real-time PCR (*Ascaris lumbricoides*, *Necator americanus* or hookworm, and *Strongyloides stercoralis* or threadworm) [13].

Odds ratios for intestinal nematode infections as an explanatory variable to malaria-resistant *vs.* malaria-sensitive were computed with Stata 10® software (College Station, Texas).

Odds ratios for intestinal nematode infections as an explanatory variable to « relapses » *vs.* « no relapses» were also computed. Given the small number of observations multivariate analyses were not used.

Results

Overall 86.8% of the children were infected by one nematode: 68.1% (n=62) were infected by hookworm, 52.7% (n=48) by threadworm and 34.1% (n=31) by *Ascaris*. A total of 65% of the children had a co-infection.

Coinfections between *N. americanus* and *S. stercoralis* were more frequent than coinfections with *Ascaris*(see Figure 2). This is probably because hookworm and threadworm are present in the same environmental types due to their similar modes of transmission.

Among the 91 children who participated in the study, 84 were included. A total of 41 children were “malaria sensitive” and 43 of them were “malaria resistant” regarding the chosen group definition.

Table 1 shows that *Ascaris* was significantly more frequent in the “resistant” malaria group than in the “sensitive” one (p=0.003). No association was found between the two other nematodes and malaria.

Among the 57 children that had at least one *P. vivax* episode, 41 had one or more relapses whereas 16 children had no relapses. *Ascaris* was more frequent in children that had no relapses but this failed to reach statistical significance. Table 1 shows that relapses seemed less frequent in the *Ascaris* group. The difference was not significant but the power to detect such a difference given the sample size was only 30%.

Discussion

Despite its very low sample size, the present study, as a number of other studies [1, 3, 4, 7, 14] reviewed [15], has found a negative association between *Ascaris* and malaria in this Amazonian setting. To explain the proximal explanation of these observations between *Ascaris* and malaria, complementary immunological hypotheses have been put forward{Nacher, 2002 #40}. This reinforces the suggestion that *Ascaris* has a protective impact on both the severity and patency of clinical infections. *Ascaris lumbricoides* has often been singled out as the most significant worm presumably because it also represents the one with the largest biomass of antigenic immunomodulatory material. In addition, *Ascaris* antigens have been reported to have a particular ability to induce a strong IgE response. Hypothetical ultimate causes lie in the mutual benefits for the worm and malaria parasites to protect their host in order to reproduce more effectively. Patients co-infected with malaria and a nematode seem to have more gametocytes, fewer symptoms associated with malaria and infection of longer duration. The anemic host has an increased attractiveness of the host for the vectors presumably leading to an increase of the number of mosquito bites. This phenomenon could affect incidence and transmission.

Although the results were not statistically significant at the 5% level, there was a trend for decreased relapses in *Ascaris*-infected patients. Surely, statistical power was much too low (28%). The hypothesis is again that *Ascaris*-mediated immunomodulation may interfere with the activation of latent hypnozoites leading to the *vivax* relapses. This had never been shown before. Given the novelty and implications of such an observation, larger studies should test this hypothesis.

The weakness of the present study was its sample size. However, despite its low statistical power the present results also find that *Ascaris* was associated with less malaria, as observed by a number of studies. An interesting corollary finding was that there also seemed to be fewer *P.vivax* relapses in the *Ascaris* group which would be a novel observation with intriguing implications on the activation of latent hypnozoites. However, this observation needs to be replicated with larger sample sizes. This data from South America brings additional data to further improve the understanding of nematodes-*Plasmodium* coinfections.

Conflict of interest statement: The authors declare that there is no conflict of interest.

References

1. Nacher M, Gay F, Singhasivanon P, et al. Ascaris lumbricoides infection is associated with protection from cerebral malaria. *Parasite immunology* 2000; **22**(3): 107-13.
2. Nacher M, Singhasivanon P, Silachamroon U, et al. Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand. *The American journal of tropical medicine and hygiene* 2001; **65**(6): 834-6.
3. Brutus L, Watier L, Hanitrasoamampionona V, Razanatsoarilala H, Cot M. Confirmation of the protective effect of Ascaris lumbricoides on Plasmodium falciparum infection: results of a randomized trial in Madagascar. *The American journal of tropical medicine and hygiene* 2007; **77**(6): 1091-5.
4. Melo GC, Reyes-Lecca RC, Vitor-Silva S, et al. Concurrent helminthic infection protects schoolchildren with Plasmodium vivax from anemia. *PloS one* 2010; **5**(6): e11206.
5. Boel M, Carrara VI, Rijken M, et al. Complex Interactions between soil-transmitted helminths and malaria in pregnant women on the Thai-Burmese border. *PLoS neglected tropical diseases* 2010; **4**(11): e887.
6. Degarege A, Animut A, Legesse M, Erko B. Malaria severity status in patients with soil-transmitted helminth infections. *Acta tropica* 2009; **112**(1): 8-11.

7. Fernandez-Nino JA, Idrovo AJ, Cucunuba ZM, et al. Paradoxical associations between soil-transmitted helminths and Plasmodium falciparum infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2012; **106**(11): 701-8.

8. Hillier SD, Booth M, Muhangi L, et al. Plasmodium falciparum and helminth coinfection in a semi urban population of pregnant women in Uganda. *The Journal of infectious diseases* 2008; **198**(6): 920-7.

9. Pullan RL, Kabatereine NB, Bukirwa H, Staedke SG, Brooker S. Heterogeneities and consequences of Plasmodium species and hookworm coinfection: a population based study in Uganda. *The Journal of infectious diseases* 2011; **203**(3): 406-17.

10. Stefani A, Hanf M, Nacher M, Girod R, Carme B. Environmental, entomological, socioeconomic and behavioural risk factors for malaria attacks in Amerindian children of Camopi, French Guiana. *Malaria journal* 2011; **10**: 246.

11. Hustache S, Nacher M, Djossou F, Carme B. Malaria risk factors in Amerindian children in French Guiana. *The American journal of tropical medicine and hygiene* 2007; **76**(4): 619-25.

12. Hanf M, Stephani A, Basurko C, Nacher M, Carme B. Determination of the Plasmodium vivax relapse pattern in Camopi, French Guiana. *Malaria journal* 2009; **8**: 278.

13. Basuni M, Muhi J, Othman N, et al. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. *The American journal of tropical medicine and hygiene* 2011; **84**(2): 338-43.

14. Murray MJ, Murray AB, Murray MB, Murray CJ. Parotid enlargement, forehead edema, and suppression of malaria as nutritional consequences of ascariasis. *The American journal of clinical nutrition* 1977; **30**(12): 2117-21.

15. Nacher M. Interactions between worms and malaria: good worms or bad worms? *Malaria journal* 2011; **10**: 259.