Journal of Infection and Public Health 16 (2023) 603–610

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

Original article

Malarial and intestinal parasitic co-infections in indigenous populations of the Brazilian Amazon rainforest

Mariana Pinheiro Alves Vasconcelos [a,b], Juan Camilo Sánchez-Arcila [b], Luciana Peres [c], Paulo Sérgio Fonseca de Sousa [c], Marcelo Augusto dos Santos Alvarenga [d], Júlio Castro-Alves [e], Maria de Fatima Ferreira-da-Cruz [f], Marilza Maia-Herzog [g], Joseli Oliveira-Ferreira [b,*]

[a] Centro de Medicina Tropical de Rondônia - CEMETRON, Porto Velho, Rondônia, Brazil
[b] Laboratório de Imunoparasitologia do Instituto Oswaldo Cruz – IOC/FIOCRUZ, Rio de Janeiro, Brazil
[c] Laboratório de Hepatites Virais do Instituto Oswaldo Cruz – IOC/FIOCRUZ, Rio de Janeiro, Brazil
[d] Laboratório de Análises Clínica do Distrito Sanitário Indígena Yanomami - DSEI-Y, Boa Vista, Roraima, Brazil
[e] Instituto Nacional de Infectologia Evandro Chagas da Fundação Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, Brazil
[f] Laboratório de Pesquisa em Malária do Instituto Oswaldo Cruz – IOC/FIOCRUZ, Rio de Janeiro, Brazil
[g] Laboratório de Referência Nacional em Simulídeos, Oncocercose e Mansonelose, Coleção de Simulídeos do Instituto Oswaldo Cruz – IOC/FIOCRUZ, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 10 October 2022
Received in revised form 13 January 2023
Accepted 12 February 2023

Keywords:
Malaria
Protozoan
Helminths
Intestinal parasites
Co-infection

ABSTRACT

The Brazilian Amazon rainforest region has a significant prevalence of malarial and intestinal parasitic infections in indigenous populations, accounting for a disproportionate burden. Thus, a cross-sectional study was conducted to assess the prevalence and association between malarial and intestinal protozoan and helminth infections in four remote indigenous villages in the Brazilian Amazon Forest. A total of 430 individuals participated in the study, and *Plasmodium* infections were diagnosed by examination of thick blood smears and PCR. Stool samples 295 individuals (69%) were examined by direct smear and the Kato–Katz technique. The overall prevalence of malaria, intestinal protozoan infection, and intestinal helminth infection was 14.2%, 100%, and 39.3%, respectively. Polyparasitism was predominant (83.7%), and most infected individuals had at least two or more different species of intestinal protozoan and/or helminth parasites. The prevalence of co-infection was 49.5%, and in individuals with intestinal protozoa and helminth infections (34%), *Entamoeba. coli*, *Entamoeba histolytica*, and *Ascaris lumbricoides* were the most common parasites. In individuals with malaria and protozoa infections (10.2%), *P. vivax*, *E. coli*, and *E. histolytica* predominated, and in individuals with malaria, protozoa, and helminth infections (5.4%). *P. vivax*, *E. coli*, *E. histolytica*, and *A. lumbricoides* predominated. Intestinal polyparasitism was common in the study population, and the presence of helminths was associated with an increased number of intestinal parasitic species. However, *Plasmodium* infections were neither a risk nor a protective factor for helminth infections; the same was true for helminth infections in relation to *Plasmodium*. The high prevalence of intestinal polyparasitism with *Plasmodium* co-infections highlights the need for combining strategies that may help control both malaria and intestinal parasite and generate a health approach aligned with indigenous perspectives.

Introduction

Malaria and intestinal parasitic infections are among the most common infections worldwide and share the same geographical area in various regions of the world [1,2]. According to the World Health Organization, 241 million cases of malaria and 627,000 deaths worldwide were estimated in 2020 and approximately 1.5 billion people worldwide are infected with at least one species of soil-transmitted helminths [3,4]. Co-infection is common in most malaria-endemic countries because of the overlapping distribution of malaria and intestinal parasites [5,6].

* Corresponding author.
E-mail address: lila@ioc.fiocruz.br (J. Oliveira-Ferreira).

https://doi.org/10.1016/j.jiph.2023.02.012

The implications of concomitant infection in humans have been previously evaluated, mainly regarding the effects of intestinal helminth infections on *Plasmodium falciparum* malaria [7–9], but existing studies have reported conflicting findings. Some studies have reported an increase in malaria parasite prevalence, parasite density, and the risk of severe disease in children [10–12]. However, some helminths species were reported to provide protection against malaria severity and related clinical outcomes of co-infected patients [7, 13, 14]. Additionally, some studies could not identify any effect of helminth and *P. falciparum* co-infection [15,16]. Data on the prevalence of intestinal protozoa co-infection are scarce, and the effect of intestinal protozoa on malaria is still unknown [17].

In Brazil, malarial and intestinal parasites and helminth infections are highly prevalent in the Amazon rainforest region, and indigenous communities are particularly vulnerable to these infections [18–20]. In 2021, the Amazon basin accounted for 99.6% of the 139,211 malaria cases reported in the country; 45,642 of these cases were reported from the indigenous areas of the Amazon, and the Yanomami community accounted for 56% (27,087) of the indigenous cases [21]. The prevalence of intestinal parasitic infections in Brazil is estimated to reach over 60%, and these infections can occur throughout the national territory, mainly in areas with low economic development and poor sanitation, as well as poor access to health services [22]. However, the lack of a mandatory reporting system may underestimate the true burden and impact of intestinal parasitic diseases in Brazil. Most studies on different indigenous ethnic groups in the Amazon have reported a high prevalence of intestinal parasites in more than half of the population [20, 22, 23]. However, the actual epidemiological situation in these communities remains unknown because of the lack of an active surveillance program throughout the national territory. Although malarial and intestinal parasites have been reported from indigenous populations, data on the prevalence of malarial and intestinal parasite co-infection have not been documented. Therefore, the present study determined the prevalence and impact of malarial parasite, helminth, and protozoa co-infection in four indigenous Yanomami villages in the Brazilian Amazon rainforest, a region where malaria is endemic.

Methods

Study area and design

A cross-sectional study was conducted in March 2015 in four remote indigenous villages of the Yanomami community of Marari and one of the basic health units in the Yanomami indigenous territory in Brazil, located in the Amazon rainforest, Amazonas. This health unit provides primary health care to four geographically close villages: Alapusi (1°11'31.8" N 64°51'30.2" W), Castanha/Ahima (1°11'39.3" N 64°48'39.2" W), Gasolina (1°08'58.4" N 64°48'40.3" W), and Taibrapa (1°10'46.8" N 64°47'52.1" W). A complete description of these areas has been reported previously [24,25]. The study area can be reached only by boats or small planes, and natives still maintain traditional modes of subsistence and are seminomadic hunter-gatherers and rural agriculturalists. The houses in the Yanomami villages, called shabonos, consist of circular wooden constructions, which can accommodate up to 300 people. The villages lack toilets, and river water is used for bathing, drinking, and cooking.

Blood sample collection and malaria diagnosis

Blood samples were collected by venipuncture using vacuum blood systems in 5 mL tubes containing EDTA anticoagulant (BD Vacutainer®). Thick blood smears (TBS) were prepared and stained with 10% Giemsa solution in a phosphate buffer solution (pH 7.2). TBS were examined using light microscopy at a health facilities in Marari by two skilled microscopists according to the malaria diagnosis guidelines of the Brazilian Ministry of Health [26]. A few drops of blood were placed on a Whatman FTA™ classic card (GE Healthcare Life Sciences) for dried blood spots (DBS). Then, the DBS were stored in zip-lock plastic bags with desiccants and transported to FIOCRUZ for malaria diagnosis using PCR. Parasite genomic DNA was extracted from the DBS of field samples on filter paper using a commercial extraction kit (QIAamp DNABlood Mini Kit; Qiagen). Screening for *Plasmodium* spp. infection was carried out using conventional and real-time PCRs with genus- and species-specific primers, as previously described [18, 27, 28]. Malaria diagnosis was evaluated in all the samples (n = 430) by TBS and PCR. Subjects were considered positive for malaria if the TBS and/or PCR results were positive. All positive cases were treated for malaria according to the treatment guidelines of the Brazilian Ministry of Health [29].

Stool sample collection and determination of intestinal parasitic infection

All individuals were provided wide mouth screw-capped containers. Parasitological examination of stool samples was performed using spontaneous sedimentation and Kato–Katz methods for the diagnosis of helminths and the direct method for the diagnosis of protozoa [30,31]. The stool samples were examined under a microscope, and two experienced professionals performed all examinations. All the patients diagnosed with intestinal parasites were subjected to treatment.

Statistical analysis

We used absolute and relative frequencies, means, and standard deviations to describe the demographic, clinical, and laboratory characteristics of the population. A comparison of the means of quantitative variables according to categorical variables was performed using the non-parametric Mann–Whitney test. Pearson's chi-square test or Fisher's exact test was used whenever necessary to verify the association between qualitative variables. We used logistic regressions to assess the association between malaria occurrence and the independent variables *Ascaris lumbricoides* (yes/no), *Ancylostoma* spp. (hookworms) (yes/no), or helminth (yes/no), with age, sex, and location as the adjustment variables. The adjusted odds ratio (aOR) with a confidence interval of 95% was estimated. $P < 0.05$ was considered statistically significant. Data were compiled in Excel spreadsheets, and statistical analysis was conducted, and graphs were generated using SPSS software (IBM-SPSS Inc., Chicago, IL, USA), the statistical software R version 4.0.2, and Graph Pad PRISM® version 8.0.

Results

Study population characteristics and prevalence of malaria and intestinal parasites

A total of 430 individuals were included in the study from four villages of the Yanomami community of Marari: 78 from Alapusi, 126 from Castanha/Ahima, 105 from Gasolina, and 121 from Taibrapa. The mean age of the participants was 31 ± 17 years, and more than 75% of the individuals were younger than 45 years. However, no differences were observed in age and sex between the villages (Table 1).

All TBS positive samples were also PCR positive; however, only 10 out of 61 PCR positive samples were TBS positive, and all mixed infections were only detected by PCR. The prevalence of malaria was 2.3% according to TBS and 14.2% according to PCR. The detection rate of *Plasmodium* using PCR was six times higher than that using TBS. The prevalence of malaria in the villages was low, except for Taibrapa

Table 1
Demographic, epidemiological, clinical, and parasitological data of individuals from the Yanomami villages of the Marari community, Amazonas, Brazil.

		Yanomami villages				
		Alapusi n = 78 n (%)	Castanha/Ahima n = 126 n (%)	Gasolina n = 105 n (%)	Taibrapa n = 121 n (%)	Total n = 430 n (%)
Sex	Male	31 (39.7)	62 (49.2)	39 (37.1)	48 (39.7)	180 (41.9)
	Female	47 (60.3)	64 (50.8)	66 (62.9)	73 (60.3)	250 (58.1)
Mean age (Mean ± SD)		31 ± 17.4	32 ± 16.7	31 ± 17.5	30 ± 17.5	31 ± 17.2
Age group	≤ 15	19 (24.4)	17 (13.5)	24 (22.9)	34 (28.1)	94 (21.9)
	> 15 ≤ 30	27 (34.6)	51 (40.5)	36 (34.3)	36 (29.8)	150 (34.9)
	> 30 ≤ 45	15 (19.2)	26 (20.6)	21 (20.0)	28 (23.1)	90 (20.9)
	> 45 ≤ 60	10 (12.8)	20 (15.9)	16 (15.2)	13 (10.7)	59 (13.7)
	> 60	6 (7.7)	8 (6.3)	8 (7.6)	10 (8.3)	32 (7.4)
	Missing	1 (1.3)	4 (3.2)	0 (0.0)	0 (0.0)	5 (1.2)
Symptoms	Headaches	7 (8.9)	2 (1.6)	6 (5.7)	6 (4.9)	21 (4.9)
	Abdominal pain	22 (28.2)	56 (44.4)	39 (37.1)	36 (29.7)	153 (35.6)
	Diarrhea	0	3 (2.4)	15 (14.3)	6 (4.9)	24 (5.6)
	Hepatomegaly	0	1 (0.8)	1 (0.9)	2 (1.6)	4 (0.9)
	Splenomegaly	0	1 (0.8)	3 (2.8)	1 (0.8)	5 (1.2)
	Asymptomatic	50 (64.1)	68 (53.9)	54 (51.4)	76 (62.8)	248 (57.7)
Malaria diagnosis	TBS	1 (1.3)	3 (2.4)	2 (1.9)	4 (3.3)	10 (2.3)
	PCR	5 (6.4)	10 (7.9)	10 (9.5)	36 (29.7)	61 (14.2)
***Plasmodium* species**	Pf	0	3 (30.0)	5 (50.0)	6 (16.6)	14 (23.0)
	Pv	4 (80.0)	3 (30.0)	4 (40.0)	17 (47.2)	28 (45.9)
	Pm	1 (20.0)	0	0	1 (2.9)	2 (3.3)
	P	0	1 (10.0)	0	0	1(1.6)
	Mixed (Pfv, Pfm, Pvm)	0	3 (30.0)	1 (10.0)	12 (33.3)	16 (26.2)
Stool samples		n = 54	n = 60	n = 90	n = 91	n = 295
IP	Protozoa only	30 (55.6)	28 (46.7)	67 (74.4)	54 (59.3)	179 (60.7)
	Protozoa + helminth	24 (44.4)	32 (53.3)	23 (25.6)	37 (40.7)	116 (39.3)

(n): absolute numbers; %: relative frequencies; TBS: thick blood smear; PCR: polymerase chain reaction; *Plasmodium* species: positives by TBS and/or PCR; Pf: P. falciparum; Pv: P. vivax; Pfv: P. falciparum and P. vivax; Pvm: P. vivax and P. malariae; P: infections that could only be diagnosed as *Plasmodium* genus positive; IP: intestinal parasites.

(29.7%), this village concentrated more than half of all malaria cases (59%). Among those with malaria, 63.9% were female, and the mean age was 34 years (Table 1).

Most infections were caused by *P. vivax* (45.9%), followed by *P. falciparum* (23.0%); mixed infections (26.2%) and infections in which only the genus *Plasmodium* could be detected (1.6%). Thus, 49.2% of the population had either *P. falciparum* or mixed infections. *P. falciparum* was prevalent in all villages, except Alapusi, and *P. malariae* was detected for the first time from this area in Ahima/Castanha, Gasolina, and Taibrapa in mono or mixed infections. Taibrapa also had the highest proportion of mixed infections (12/16) (Table 1). Most individuals were asymptomatic (57.7%), and among those with symptoms, abdominal pain (35.6%) was the main complaint. The prevalence of malaria among symptomatic (n = 182) and asymptomatic (n = 248) individuals was similar, 11% and 16.5% (p = 0.09), respectively. In individuals with symptomatic malaria (n = 20), 44% had at least one of the symptoms associated with malaria and the most prevalent symptom was headache (9.8%) while abdominal pain was the most frequent symptom among the individuals negative for malaria. Three subjects had fever, but none of them were diagnosed with malaria and the fever was attributed to other causes such as tonsillitis and pneumonia.

Intestinal parasites were evaluated in stool samples of 295 individuals. Overall, 100% of the population had intestinal protozoa infection, and 39.3% had co-infections of protozoa and helminths. The distribution of the intestinal parasitic species detected from each village is shown in Fig. 1. In the 295 individuals with intestinal parasitic infection, 11 parasitic species were detected: five protozoa and six helminths. The most common species of protozoa detected in all villages was *Entamoeba coli* (100%), followed by *E. histolytica* (71.5%), *Iodamoeba bütschlii* (33.2%), and *Giardia intestinalis* (6.4%). Regarding the helminth species, hookworms and *A. lumbricoides* (19.7%) were most prevalent (20.4%) in all villages. *Trichuris trichiura* (3.7%) and *Enterobius vermicularis* (1.4%) were also present in all villages except Taibrapa. *Strongyloides stercoralis* (0.7%) was present only in Castanha/Ahima and *Hymenolepis nana* (2.0%), in Gasolina and Taibrapa. The prevalence of intestinal helminths was 53.3% in Castanha/Ahima, 44.4% in Alapusi, 40.7% in Taibrapa, and 25.6% in Gasolina. Symptoms associated with intestinal parasites, such as abdominal pain and diarrhea, were present in 35.6% and 5.6%, respectively. However, none of these symptoms were associated with the presence of helminths (p > 0.05).

Malarial and intestinal parasites co-infections

The frequency of single and multiple parasitic infections was analyzed in 295 individuals who underwent blood and stool examinations. As shown in Table 2, they were grouped according to their infection status: P, individuals infected with intestinal protozoa only (50.5%); P + H, individuals co-infected with intestinal protozoa and helminths (33.9%); P + M, individuals co-infected with intestinal protozoa and malarial parasite (10.2%); and P + H+M, individuals co-infected with intestinal protozoa, helminths, and malarial parasite (5.4%). Polyparasitism was predominant in all villages, and most of the infected individuals had two or more species of intestinal parasites. However, the prevalence of M+P or M+P + H co-infections was higher in Taibrapa than in other villages (p < 0.01).

Among the individuals with intestinal parasites, 16.3%, 33.2%, 30.1%, and 15.6% had single, double, triple, and quadruple parasite species of protozoa and/or helminths, respectively (Table 2). Although not statistically significant, individuals co-infected with *Plasmodium* had fewer intestinal parasite species (2.33 *versus* 2.67 parasites, p > 0.05) than those without *Plasmodium* infection. Among the individuals co-infected with M+P, 76.7% were female, with a mean age of 34.1 years, whereas among the individuals with M+P + H triple infection, 43.7% were female, with a mean age of 29.7 years.

Polyparasitism with helminths was significantly associated with a greater number of intestinal parasite species (3.48 *versus* 2.03 parasites, p < 0.001), but no difference between males and females was observed.

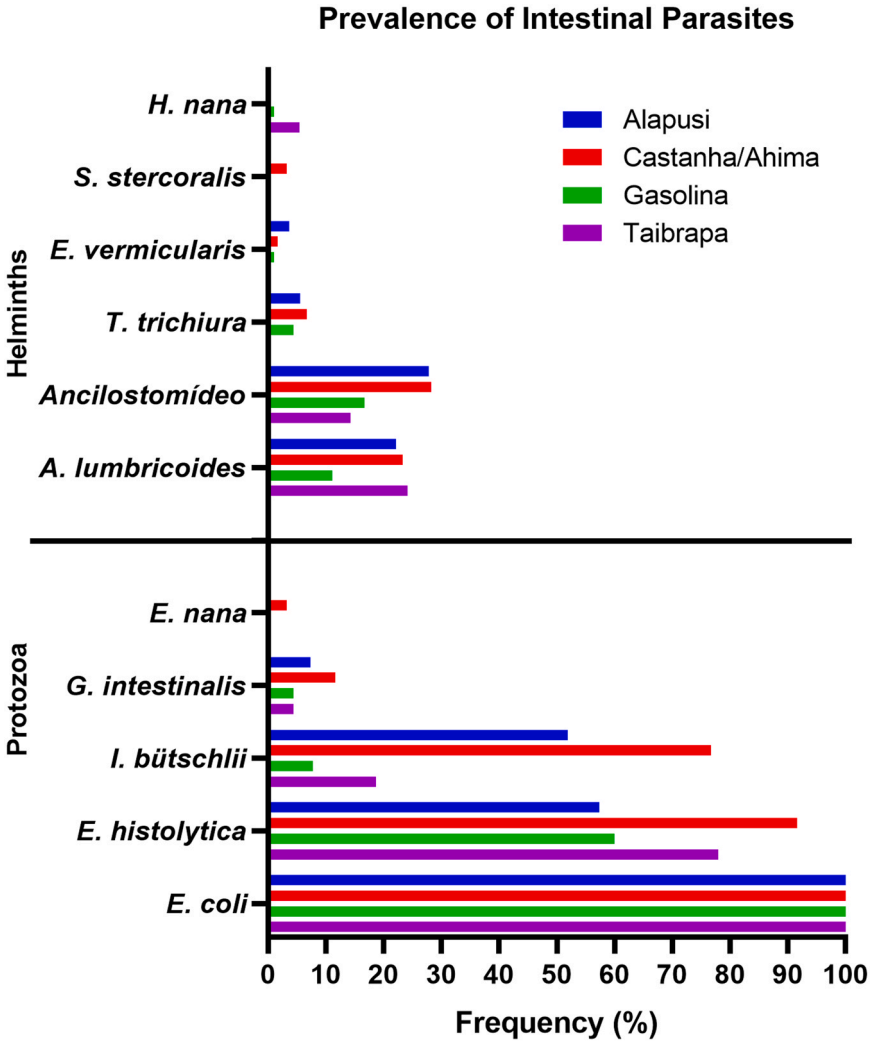

Fig. 1. Distribution of intestinal protozoan and helminthic parasites in individuals from the Yanomami villages at Marari community, Amazonas, Brazil.

Table 2
Absolute (n) and relative (%) frequency of the infection status of individuals by village and number of intestinal parasites at Marari community, Amazonas, Brazil.

	Infection Status				
	P	P + H	P + M	P + H+M	Total
	n = 149 n (%)	**n = 100** n (%)	**n = 30** n (%)	**n = 16** n (%)	**n = 295** n (%)
Villages					
Alapusi	28 (18.8)	22 (22.0)	2 (6.7)	2 (12.5)	54 (56.8)
Castanha/ Ahima	26 (17.5)	30 (30.0)	2 (6.7)	2 (12.5)	60 (20.3)
Gasolina	61 (40.9)	20 (20.0)	6 (20.0)	3 (18.8)	90 (30.5)
Taibrapa	34 (22.8)	28 (28.0)	20 (66.6)	9 (56.2)	91 (30.8)
Total	149 (50.5)	100 (33.9)	30 (10.2)	16 (5.4)	295 (100)
No of IP species					
1	40 (26.8)	0	8 (26.7)	0	48 (16.3)
2	66 (44.3)	12 (12.0)	17 (56.7)	3 (18.7)	98 (33.2)
3	37 (24.8)	39 (39.0)	4 (13.3)	9 (56.3)	89 (30.1)
4	6 (4.1)	35 (35.0)	1 (3.3)	4 (25.0)	46 (15.6)
≥ 5	0	14 (14.0)	0	0	14 (4.8)

IP: intestinal parasites; P: only protozoa infection; P + H: intestinal protozoa and helminth co-infection; P + M: intestinal protozoa and malarial parasite co-infection; P + H+M: intestinal protozoa, helminth, and malaria co-infection.

The heatmap in Fig. 2 shows the distribution of intestinal parasitic and *Plasmodium* species in each village. Considering the infection status of the individuals, the highest number of parasitic species combinations was observed in the P + H group with 34 possible combinations, followed by M+P + H (13 combinations), M+P (11 combinations), and P (7 combinations). Despite the low number of different species combinations in the P group, protozoa accounted for the highest number of cases observed in the population. In particular, infections caused by *E. coli* and *E. coli* + *E. histolytica* were the most abundant parasitic infections in Alapusi, Taibrapa, and Gasolina. Co-infection *with E. coli* + *E. histolytica* + *I. butschlii* was particularly abundant in Ahima/Castanha, whereas *E. coli* + *E. histolytica* + *A. lumbricoides* co-infection was observed in Taibrapa. Overall, the prevalence of P + M and P + H+M confection was low in all villages, except Taibrapa, where P + M and P + H+M malaria co-infection were more frequent.

With respect to age distribution (Fig. 3), when we compared the group of individuals with protozoan only (P) and the group with protozoa and helminths, the mean age in this group was significantly lower than the group with protozoa only (mean age: 28 *versus* 34 years old, $p = 0.03$). However, no differences were observed with the other groups, despite the higher mean age in the group P + M ($p > 0.05$).

Logistic regression analysis was also performed to assess the association between malaria occurrence and the independent variables, such as helminths, *A. lumbricoides*, or *Ancylostoma* sp., with age, sex, and villages as the adjustment variables. No association was observed between malarial parasite and helminth, *A. lumbricoides*, or hookworm co-infections. The results of the final model are summarized in Table 3.

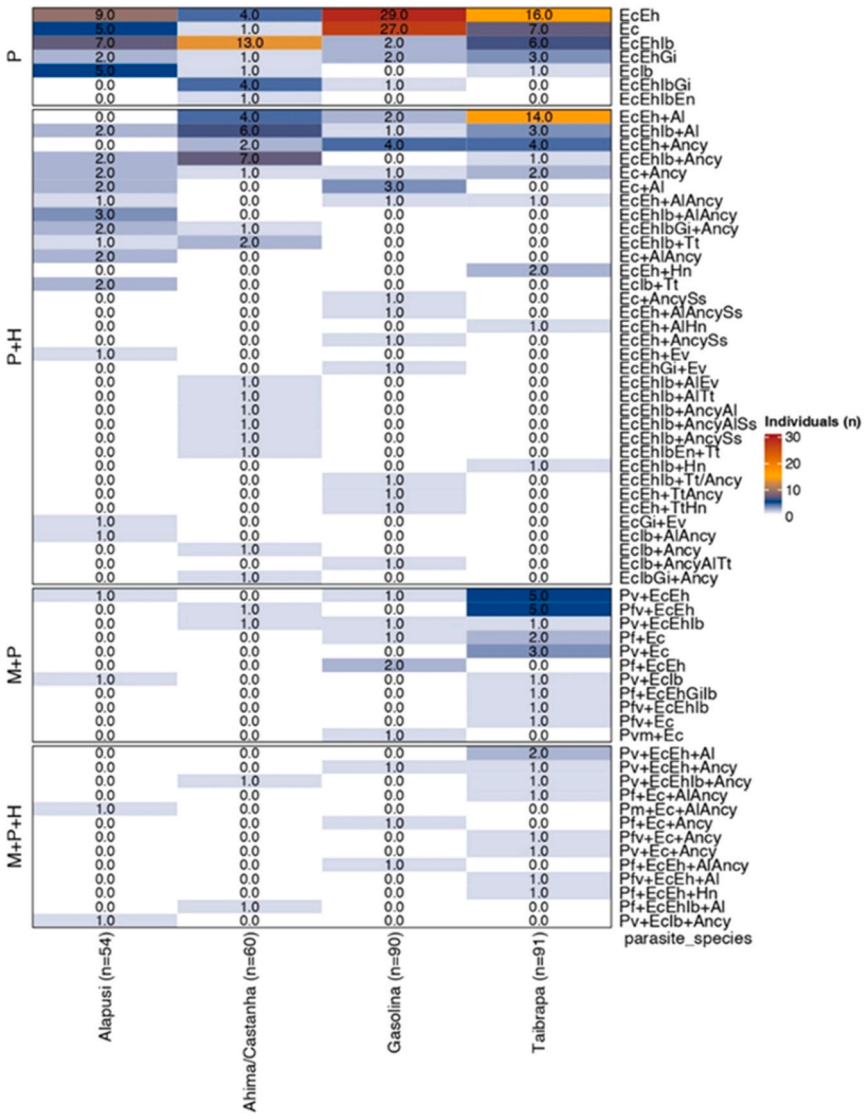

Fig. 2. Heatmap of intestinal and malarial parasite co-infections in individuals from Yanomami villages at Marari community, Amazonas, Brazil. M: malaria; P: protozoa; H: helminth. Pf: *P. falciparum*; Pv: *P. vivax*; Pfv: *P. falciparum* and *P. vivax*; Pvm: *P. vivax* and *P. malariae*; Ec: *E. coli*; Eh: *E. histolytica*; Ib: *I. bütschlii*; Gi: *G. intestinalis*; En: *E. nana*; Al: *A. lumbricoides*; Tt: *T. trichiura*; Hn: *H. nana*; Ev: *E. vermicularis*; Ss: *S. stercoralis*; Ancy: *Ancylostoma* (hookworms).

Discussion

Although malarial and intestinal parasites share the same geographical area in Brazil and are common in many indigenous communities in the Amazon, few studies have reported the association between the diseases caused by them. Thus, we performed a cross-sectional study and surveyed malarial and intestinal parasitic infections. The results demonstrated that the likelihood of malaria infection was significantly higher in individuals infected with intestinal parasites in the study villages, since all individuals were infected with intestinal protozoa and 83.7% were infected with at least two species of protozoa and/or helminths.

We found that 15.6% of the individuals were infected with *Plasmodium*, 37% with one or more species of helminths, and 100% with at least one protozoan. The high prevalence of intestinal parasites in all villages indicated a great diversity of parasitic species, high infection rates, and mixed parasitosis, suggesting that the individuals were frequently exposed to human fecal contamination.

Most of the subjects with malaria were asymptomatic, and malaria infection was not associated with fever in any individual, even in the younger age groups, which was probably because of low parasitemia. In fact, malaria infection was diagnosed by TBS in only 2.3% of the individuals, in contrast to PCR, which diagnosed 13.5%. Using PCR, which is more sensitive than TBS, approximately 86% of infections were found to be submicroscopic in this semi-isolated group and diagnosed only by PCR. Similar results were observed in our previous 4-month follow-up study in the same villages [32]. These results are relevant, as asymptomatic carriers remain untreated and are sources of infection for *Anopheles*, which maintains the life cycle and transmission chain of the parasite in Yanomami villages [36]. Our findings highlight the importance of molecular diagnosis in estimating the prevalence of malaria in indigenous endemic areas of the Amazon.

The prevalence of protozoa infections, such as those caused by *G. intestinalis* and *Entamoeba* spp., in the indigenous populations of Brazil is generally high, varying according to the indigenous ethnic groups, such as 66.5% in the Terena [33], 70.7% in the Surui [34], 89,5% in the Maxakali [35] and 65% in the Parakanã [24]. However, to the best of our knowledge, not all intestinal protozoan infections have been described yet. In the present study, *E. histolytica* and *E. coli* were found in 71.0% and 100% of the individuals, respectively. Intestinal protozoa may affect hundreds of millions of people annually.

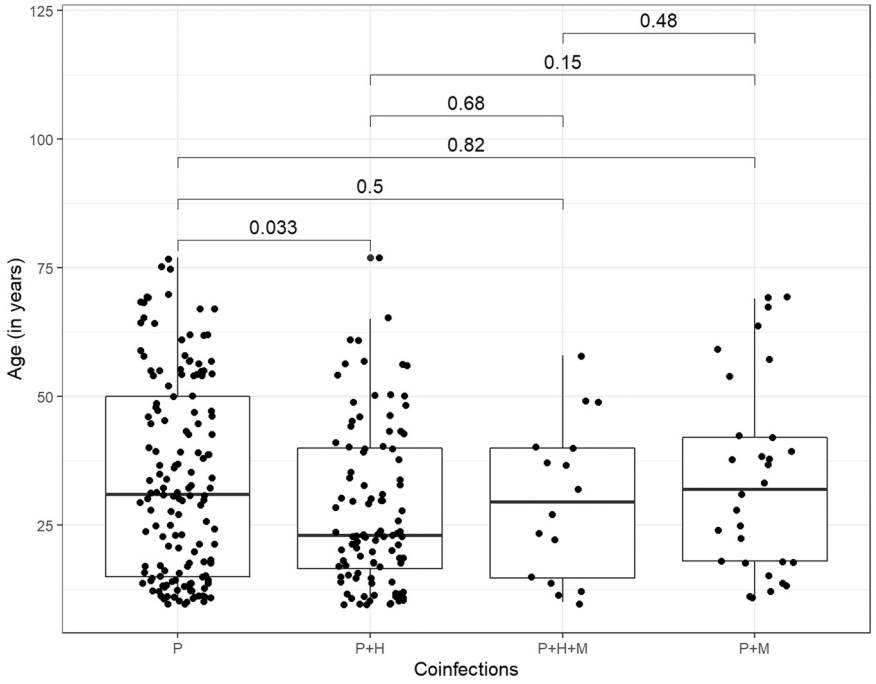

Fig. 3. Malaria and intestinal parasites by age. Mean age of individuals infected with intestinal protozoa monoinfection (P) or co-infected with helminths (P + H) or malaria and/or helminths (P + H+M and P + M). A statistically significant difference was observed between monoinfected protozoan and co-infected protozoan and helminth groups ($p = 0.03$). All p values are shown in the figure and $p > 0.05$ was not considered significant.

Table 3
Results of the adjusted logistic models to assess the association of the occurrence of malarial and intestinal parasites in individuals from Yanomami villages at the Marari community, Amazonas, Brazil.

	Malaria			
Intestinal parasites	No (n = 249)	Yes (n = 46)	aOR [95%CI]	*p*
Helminth				
No	149 (59.8)	30 (65.2)	0.81 [0.3;1.6]	0.56
Yes	100 (40.2)	16 (34.8)		
Ascaris lumbricoides				
No	198 (79.5)	39 (84.8)	0.58 [0.2;1.4]	0.25
Yes	51 (20.5)	7 (15.2)		
Hookworm				
No	200 (80.3)	35 (76.1)	1.89 [0.8;4.2]	0.13
Yes	49 (19.7)	11 (23.9)		

However, there are no reliable estimates of the global burden of intestinal protozoan diseases, and until now, the interaction between malarial and intestinal protozoa had not been adequately described. The prevalence of helminths (39.3%) in our study individuals was low compared with that of other indigenous populations in the Brazilian Amazon, in which *A. lumbricoides* and hookworms (45–95%), as well as protozoa such as *Entamoeba* spp. and *G. intestinalis*, are predominant [20, 36–38]. Although more severe forms of amoebiasis and giardiasis are rare, both *E. histolytica* and *G. duodenalis* can cause acute and persistent diarrhea and may be implicated in nutrient malabsorption, leading to malnutrition, growth and development retardation [39–41].

The prevalence of malarial and intestinal parasitic co-infection (15.6%) in our study was higher than that observed in a rural area of Rondonia, Brazil [25] but lower than that observed in Oiapoque, Amapa [42] and some African and Asian regions [10–14]. In the individuals co-infected with malarial and intestinal protozoa, the prevalence of co-infection with *P. vivax*, *E. coli*, and *E. histolytica* predominated, whereas in individuals co-infected with malarial and intestinal protozoa and helminths, *P. vivax, E. coli*, *E. histolytica*, and *A. lumbricoides* were predominant. To the best of our knowledge, the present study is the first to report malarial and intestinal parasitic co-infection in an indigenous community of the Brazilian Amazon.

In our study we did not show a significant association between malarial and intestinal parasitic infections, contrary to studies from the endemic regions of Africa and Asia [6, 7, 9–11, 20, 43]. This difference may be related to the *Plasmodium* species, intestinal parasitic infections, and the age of the study population. While most of the co-infection studies in malaria endemic areas of Africa are with *P. falciparum* and helminths in children, in our study, *P. vivax* co-infection was predominant and all individuals co-infected with helminths were also co-infected with protozoa and the age of the participants was above 9 years. In addition, our data were analyzed after combining different Plasmodium, protozoan, and helminth species into a single group, since the large variety of *Plasmodium* and intestinal parasitic species did not form groups with large sample sizes to evaluate the effect of coinfection. Additionally, for logistic reasons, a single stool sample was collected from each individual in the present study, which may have caused an underestimation of the prevalence of intestinal helminth infections.

A study in Thailand showed that the incidence of malaria was positively associated with hookworm infections but negatively associated with *A. lumbricoides* when helminths were stratified by species [44]. However, this relationship was not maintained when the data were analyzed after pooling different helminth species into a single group [44].

In the Brazilian Amazon region one study reported the association of intestinal helminthiases with protection against reduced hemoglobin levels during *P. vivax* malaria episodes in a population of children in the city of Manaus [45]. However, in Rondônia, co-infection with enteroparasites did not affect the immune response pattern to *P. vivax* malaria [25,46], but differences were observed between the hemoglobin levels of malaria patients and individuals who were not infected by enteroparasites on the Brazil and French Guiana border [42].

In our study, we did not observe a significant association between malarial and intestinal parasitic infections. However, the high prevalence of intestinal parasites and co-infection by 1–5 species of

parasites in indigenous population, are especially important due to negative effects in nutrient digestion and absorption, threatening not only growth potential, but also raising the risk of death in indigenous children [47]. The critical nutritional status of the Yanomami children living in Brazil and Venezuela has been denounced for at least three decades [40, 48–50].

The precarious housing conditions, inadequate sanitation, environmental contamination, and contaminated food and water in the Yanomami villages are factors that may contribute to the year-round transmission of intestinal parasites and may help to understand not only the elevated exposure of these population to infectious and parasitic diseases, but the severity of the nutritional situation in children. Data from anthropometric surveys in more than 50 countries worldwide suggest that, to reduce undernutrition, deworming programs should be implemented before 24 months of age [51]. However, drug mass therapy may reduce morbidity in the short run but it does not ensure sustained control of intestinal parasitism. This can only be achieved by reducing both fecal contamination of the environment and human exposure to potentially contaminated sources.

Conclusion

Intestinal parasitic infections are highly prevalent, along with malaria, in Yanomami villages, but we could not find an association between malarial and intestinal parasites. However, the high prevalence of intestinal polyparasitism identified in this study indicates that environmental contamination with infective parasite forms is critical to the epidemiology of intestinal parasitism. For this reason, housing and sanitation play a key role in control strategies. Educational interventions to promote better hygiene and periodic de-worming aimed are considered important supplementary measures. A sustainable program for the control of intestinal parasites in the Yanomami villages should consider alternative ways for environmental sanitation that consider indigenous perceptions about gastrointestinal disease, housing, personal hygiene, and environmental health.

Funding

This work was majorly supported by Instituto Oswaldo Cruz -IOC/FIOCRUZ and Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq (479559/2013), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance code 001, PAEF/FIOCRUZ (IOC-FIO-18–2–47), FAPERJ -Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro E-26/203.133/2019 and E-26/200.938/2022. The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Ethical approval

The study protocol was approved by the Brazilian National Research Ethics Commission (CONEP, Protocol #16907), which regulates studies involving indigenous Brazilian populations and was locally supervised by their representatives. Participants of the study were required to meet the following criteria: (i) above 10 years, (ii) sign the consent form, and (iii) consent to blood collection. The recruitment of volunteers in the Marari community involved a bilingual interpreter who explained to the leaders and/or indigenous representatives the purpose of the study, the procedures to be conducted, and finally requested verbal and written consent from each participant or the guardians on behalf of minors prior to their inclusion in the study. Two physicians among the authors (Drs. M.P.A. Vasconcelos and L. Peres) performed the physical examinations. Epidemiological data, such as age, sex, and previous malaria infection, were obtained during the interviews.

Acknowledgements

The authors would like to thank the Yanomami people for participating in this study and for their help in many ways during the fieldwork, the Secretaries Especial de Saúde Indígena (SESAI), and Distrito Sanitário Especial Indígena Yanomami (DSEI-Y) and their health professionals for logistic support and help in the fieldwork.

Author's contribution

Conceived, designed, and drafted the manuscript: JOF, MPAV, MMH. Investigated and performed the experiments: JOF, MPAV, JCSA, LP, PSFS, MASA, MFFC. Analyzed the data: MPA, JCSA, JCSA. Contributed reagents/materials/analysis tools: MFFC, MMH, JCA. All authors have read and approved the final manuscript.

References

[1] Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit Vectors 2014;7:37. https://doi.org/10.1186/1756-3305-7-37
[2] Hotez PJ, Alvarado M, Basanez MG, Bolliger I, Bourne R, Boussinesq M, et al. The global burden of disease study 2010: interpretation and implications for the neglected tropical diseases. PLoS Negl Trop Dis 2014;8:e2865. https://doi.org/10.1371/journal.pntd.0002865
[3] WHO. Soil-transmitted helminth infections 〈https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections〉 ; 2022.
[4]. WHO. World malaria report 2020: 20 years of global progress and challenges. Geneva: World Health Organization; 2020. Contract No.: CC BY-NC-SA 3.0 IGO.
[5] Afolabi MO, Ale BM, Dabira ED, Agbla SC, Bustinduy AL, Ndiaye JLA, et al. Malaria and helminth co-infections in children living in endemic countries: a systematic review with meta-analysis. PLoS Negl Trop Dis 2021;15:e0009138. https://doi.org/10.1371/journal.pntd.0009138
[6] Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, et al. Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. Am J Trop Med Hyg 2007;77:88–98.
[7] Nacher M, Singhasivanon P, Silachamroon U, Treeprasertsuk S, Vannaphan S, Traore B, et al. Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand. Am J Trop Med Hyg 2001;65:834–6. https://doi.org/10.4269/ajtmh.2001.65.834
[8] Babamale OA, Ugbomoiko US, Heukelbach J. High prevalence of Plasmodium falciparum and soil-transmitted helminth co-infections in a periurban community in Kwara State, Nigeria. J Infect Public Health 2018;11:48–53. https://doi.org/10.1016/j.jiph.2017.03.002
[9] Degarege A, Legesse M, Medhin G, Animut A, Erko B. Malaria and related outcomes in patients with intestinal helminths: a cross-sectional study. BMC Infect Dis 2012;12:291. https://doi.org/10.1186/1471-2334-12-291
[10] Degarege A, Animut A, Legesse M, Erko B. Malaria severity status in patients with soil-transmitted helminth infections. Acta Trop 2009;112:8–11. https://doi.org/10.1016/j.actatropica.2009.05.019
[11] Sokhna C, Le Hesran JY, Mbaye PA, Akiana J, Camara P, Diop M, et al. Increase of malaria attacks among children presenting concomitant infection by Schistosoma mansoni in Senegal. Malar J 2004:3. https://doi.org/10.1186/1475-2875-3-43
[12] Spiegel ATA, Raphenon G, Trape JF, Druilhe P. Increased frequency of malaria attacks in subjects co-infected by intestinal worms and Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg 2003;97:198–9.
[13] Lemaitre M, Watier L, Briand V, Garcia A, Le Hesran JY, Cot M. Coinfection with Plasmodium falciparum and Schistosoma haematobium: additional evidence of the protective effect of Schistosomiasis on malaria in Senegalese children. Am J Trop Med Hyg 2014;90:329–34. https://doi.org/10.4269/ajtmh.12-0431
[14] Nacher M, Singhasivanon P, Traore B, Vannaphan S, Gay F, Chindanond D, et al. Helminth infections are associated with protection from cerebral malaria and increased nitrogen derivatives concentrations in Thailand. Am J Trop Med Hyg 2002;66:304–9. https://doi.org/10.4269/ajtmh.2002.66.304
[15] Shapiro AE, Tukahebwa EM, Kasten J, Clarke SE, Magnussen P, Olsen A, et al. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. Trans R Soc Trop Med Hyg 2005;99:18–24. https://doi.org/10.1016/j.trstmh.2004.02.006
[16] Ojurongbe O, Adegbayi AM, Bolaji OS, Akindele AA, Adefioye OA, Adeyeba OA. Asymptomatic falciparum malaria and intestinal helminths co-infection among school children in Osogbo, Nigeria. J Res Med Sci 2011;16:680–6.
[17] Geus D, Sifft KC, Habarugira F, Mugisha JC, Mukampunga C, Ndoli J, et al. Co-infections with Plasmodium, Ascaris and Giardia among Rwandan school-children. Trop Med Int Health 2019;24:409–20. https://doi.org/10.1111/tmi.13206

[18] Zalis MG, Ferreira-da-Cruz MF, Balthazar-Guedes HC, Banic DM, Alecrim W, Souza JM, et al. Malaria diagnosis: standardization of a polymerase chain reaction for the detection of Plasmodium falciparum parasites in individuals with low-grade parasitemia. Parasitol Res 1996;82:612–6. https://doi.org/10.1007/s004360050173
[19] Meireles BM, de Souza Sampaio V, Monteiro WM, Goncalves MJF. Factors associated with malaria in indigenous populations: A retrospective study from 2007 to 2016. PLoS One 2020;15:e0240741. https://doi.org/10.1371/journal.pone.0240741
[20] Ferrari JO, Ferreira MU, Camargo LMA, Ferreira CS. Intestinal Parasites among Karitiana Indians from Rondonia State, Brazil. Rev I. Med Trop 1992;34:223–5. https://doi.org/10.1590/S0036-46651992000300007
[21] Ministério da Saúde B. Information System for Epidemiological Surveillance - Malaria (SIVEP-Malaria) Available from: 〈htpp//portalweb04.saude.gov.br/Sivep_malaria/〉 [Internet]. 2022.
[22] Celestino AO, Vieira SCF, Lima PAS, Rodrigues L, Lopes IRS, Franca CM, et al. Prevalence of intestinal parasitic infections in Brazil: a systematic review. Rev Soc Bras Med Trop 2021;54:e00332021. https://doi.org/10.1590/0037-8682-0033-2021
[23] Genaro O, Ferraroni JJ. [Malaria and intestinal parasitosis in Indians of the Nadeb-Maku tribe, State of Amazonas, Brazil]. Rev Saude Publica 1984;18:162–9. https://doi.org/10.1590/s0034-89101984000200008
[24] Miranda RA, Xavier FB, Menezes RC. [Intestinal parasitism in a Parakana indigenous community in southwestern Para State, Brazil]. Cad Saude Publica 1998;14:507–11.
[25] Sánchez-Arcila JC, DdS Perce-da-Silva, Vasconcelos MPA, Rodrigues-da-Silva RN, Pereira VA, Aprígio CJL, et al. Intestinal Parasites Coinfection Does Not Alter Plasma Cytokines Profile Elicited in Acute Malaria in Subjects from Endemic Area of Brazil. Mediat Inflamm 2014;2014:857245. https://doi.org/10.1155/2014/857245
[26] Brasil. Manual de Diagnóstico da Malária. In: Brasilia MdSSdVeS, editor. 〈https://bvsms.saude.gov.br/bvs/publicacoes/manual_diagnostico_laboratorial_malaria_2ed.pdf2009〉 .
[27] Almeida-de-Oliveira NK, Moreira OC, de Lavigne AR, Mendonca-Lima L, Werneck GL, Daniel-Ribeiro CT, et al. Analytical validation of real-time quantitative PCR assays for optimum diagnosis of vivax malaria. Mem Inst Oswaldo Cruz 2019;114:e180350. https://doi.org/10.1590/0074-02760180350
[28] Snounou G, Singh B. Nested PCR analysis of Plasmodium parasites. Methods Mol Med 2002;72:189–203. https://doi.org/10.1385/1-59259-271-6:189
[29] Brasil. Guia pratico de tratamento da malaria no Brasil. In: Saude. SdVe, editor. Brasilia.2021.
[30] WHO. Bench Aids for the Diagnosis of Intestinal Parasites. May 2019; 2nd Edition.
[31] Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thicksmear technique in Schistosomiasis mansoni. Rev I Med Trop 1972:397–400.
[32] Robortella DR, Calvet AA, Amaral LC, Fantin RF, Guimaraes LFF, Franca Dias MH, et al. Prospective assessment of malaria infection in a semi-isolated Amazonian indigenous Yanomami community: transmission heterogeneity and predominance of submicroscopic infection. PLoS One 2020;15:e0230643. https://doi.org/10.1371/journal.pone.0230643
[33] Aguiar JI, Gonçalves AQ, Sodré FC, Pereira Sdos R, Bóia MN, de Lemos ER, et al. Intestinal protozoa and helminths among Terena Indians in the State of Mato Grosso do Sul: high prevalence of Blastocystis hominis. Rev Soc Bras Med Trop 2007;40:631–4. https://doi.org/10.1590/s0037-86822007000600006
[34] Palhano-Silva CS, Araujo AJG, Lourenco AEP, Bastos OMP, Santos RV, Coimbra CEA. Intestinal parasitic infection in the Surui Indians, Brazilian Amazon. Interciencia 2009;34:259–64.
[35] Assis EM, Olivieria RC, Moreira LE, Pena JL, Rodrigues LC, Machado-Coelho GL. Prevalence of intestinal parasites in the Maxakali indigenous community in Minas Gerais, Brazil, 2009. Cad Saude Publica 2013;29:681–90. https://doi.org/10.1590/s0102-311×2013000800006
[36] Santos RV. Coimbra Junior CE, Flowers NM, Silva JP. Intestinal parasitism in the Xavante Indians, central Brazil. Rev Inst Med Trop Sao Paulo 1995;37:145–8. https://doi.org/10.1590/s0036-46651995000200009
[37] Lawrence DN, Neel JV, Abadie SH, Moore LL, Adams LJ, Healy GR, et al. Epidemiologic studies among Amerindian populations of Amazonia. III. Intestinal parasitoses in newly contacted and acculturating villages. Am J Trop Med Hyg 1980;29:530–7.
[38] Martins M, Lacerda MV, Monteiro WM, Moura MA, Santos EC, Saraceni V, et al. Progression of the load of waterborne and intestinal parasitic diseases in the State of Amazonas. Rev Soc Bras Med Trop 2015;48(Suppl 1):42–54. https://doi.org/10.1590/0037-8682-0162-2014
[39] Farthing M, editor Tropical malabsorption. Seminars in gastrointestinal disease; 2002.
[40] Lima AA, Guerrant RL. Persistent diarrhea in children: epidemiology, risk factors, pathophysiology, nutritional impact, and management. Epidemiol Rev 1992;14:222–42. https://doi.org/10.1093/oxfordjournals.epirev.a036088
[41] Fauziah N, Aviani JK, Agrianfanny YN, Fatimah SN. Intestinal parasitic infection and nutritional status in children under five years old: a systematic review. Trop Med Infect Dis 2022:7. https://doi.org/10.3390/tropicalmed7110371
[42] Menezes RAO, Gomes M, Mendes AM, Couto A, Nacher M, Pimenta TS, et al. Enteroparasite and vivax malaria co-infection on the Brazil-French Guiana border: epidemiological, haematological and immunological aspects. PLoS One 2018;13:e0189958. https://doi.org/10.1371/journal.pone.0189958
[43] Olarewaju A, Babamalea UU, Heukelbach Jorg. High prevalence of Plasmodium falciparum and soil-transmittedhelminth co-infections in a periurban community in Kwara State, Nigeria. J Infect Public Health 2018;11:48–53.
[44] Boel M, Carrara VI, Rijken M, Proux S, Nacher M, Pimanpanarak M, et al. Complex Interactions between soil-transmitted helminths and malaria in pregnant women on the Thai-Burmese border. PLoS Negl Trop Dis 2010;4:e887. https://doi.org/10.1371/journal.pntd.0000887
[45] Melo GC, Reyes-Lecca RC, Vitor-Silva S, Monteiro WM, Martins M, Benzecry SG, et al. Concurrent helminthic infection protects schoolchildren with plasmodium vivax from Anemia. Plos One 2010:5. https://doi.org/10.1371/journal.pone.0011206
[46] Sanchez-Arcila JC, Perce-da-Silva DS, Vasconcelos MP, Rodrigues-da-Silva RN, Pereira VA, Aprigio CJ, et al. Intestinal parasites coinfection does not alter plasma cytokines profile elicited in acute malaria in subjects from endemic area of Brazil. Mediat Inflamm 2014;2014:857245. https://doi.org/10.1155/2014/857245
[47] Tanner S, Chuquimia-Choque ME, Huanca T, McDade TW, Leonard WR, Reyes-García V. The effects of local medicinal knowledge and hygiene on helminth infections in an Amazonian society. Soc Sci Med 2011;72(1982):701–9. https://doi.org/10.1016/j.socscimed.2010.12.012
[48] Gendrel D, Treluyer JM, Richard-Lenoble D. Parasitic diarrhea in normal and malnourished children. Fundam Clin Pharmacol 2003;17:189–97. https://doi.org/10.1046/j.1472-8206.2003.00169.x
[49] Horta BL, Santos RV, Welch JR, Cardoso AM, dos Santos JV, Assis AMO, et al. Nutritional status of indigenous children: findings from the First National Survey of Indigenous People's Health and Nutrition in Brazil. Int J Equity Health 2013;12:23. https://doi.org/10.1186/1475-9276-12-23
[50] Verhagen LM, Incani RN, Franco CR, Ugarte A, Cadenas Y, Sierra Ruiz CI, et al. High malnutrition rate in Venezuelan Yanomami compared to Warao Amerindians and Creoles: significant Associations WITH intestinal parasites and anemia. PLOS ONE 2013;8:e77581. https://doi.org/10.1371/journal.pone.0077581
[51] Victora CG, de Onis M, Hallal PC, Blössner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. Pediatrics 2010;125:e473–80. https://doi.org/10.1542/peds.2009-1519