Prevalence of multiple concomitant intestinal parasitic infections in Simbok a malaria endemic village in Cameroon

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ABSTRACT

Little is known about the effects of concomitant intestinal parasitosis on malaria infection and immunity. An ongoing longitudinal study on acquisition of immunity to malaria in Simbok population is enabling us to address this question. From each of the 185 volunteers (children and adults) recruited for the study, about 4cm3 of stool sample was collected in a clean tube, processed with sodium chloride and Lugol iodine solutions, and examined for the presence of intestinal parasites by microscopy. Thick and thin blood smears prepared from blood collected by venepuncture and stained using Field stain method were also examined for the presence of malaria parasites by microscopy. Results obtained showed malaria and intestinal parasites to be prevalent in Simbok. Entamoeba histolytica (42.6%), Plaspodium falciparum (23.9%), Ascaris lumbricoides (23.2%) and Necator americanus (23.2%) were the most prevalent parasites in the population. Concomitant infections involving 1-4 parasite species were recorded. Most of the observed parasites were potentially pathogenic with infection level in the cases examined not high enough to be symptomatic. The mean age of subjects with concomitant E. histolytica, A. lumbricoides and malaria infections (7.55±2.03years) differed significantly from those with concomitant A. lumbricoides and E. histolytica but without malaria (17.35±14.89 years): t=2.0; P<0.05. Thus, concomitant A. lumbricoides and E. histolytica infections may down-regulate immunity to malaria in young persons. P. falciparum-infected subjects were observed to have significantly lower PCV (36.72±4.92%) than those without P. falciparum-infection (40.16±4.58%): t=4.21: P<0.05. Similarly, subjects with N. americanus had low PCV (37.9±5.08%) compared to those without (40.00±4.82 %): t=2.48; P<0.05. Thus, in endemic areas for concomitant malaria and intestinal parasites, infections with both P falciparum and Necator americanus may contribute to anaemia.

Key words: malaria infection, intestinal parasitosis, concomitant infections, immunity

RÉSUMÉ

Beaucoup reste à connaître à propos des effets des infections parasitaires concomitantes sur l'immunité contre le paludisme. Cette étude de l'acquisition de l'immunité contre le paludisme dans le village Simbok nous a permis de poser ce problème. De chacun des 185 volontaires (enfants et adultes) recrutés dans cette étude, environ 4 cm3 de selles ont été collectéss et analysées avec la solution de chlorure de sodium et de l'eau iodée; et examinées au microscope pour la recherche des parasites intestinaux. Le sang collecté par ponction veineuse a été utilisé pour la confection de la goutte épaisse et du frottis mince dans le but de rechercher des plasmodiums au microscope, après coloration par la méthode de Field. Le paludisme et les infections intestinales étaient prévalants à Simbok. Entamoeba histolytica (42.6%), P falciparum (23.9%), Ascaris lombricoides (23.2%) et Necator americanus (23.2%) étaient les parasites les plus prévalants. Les infections concomitantes impliquant 2 à 4 espèces parasitaires étaient enregistrées dans cette étude. La plupart de parasites identifiés étaient des potentiels pathogènes mais la charge parasitaire dans les cas examinés n'était pas assez élevée pour être symptomatique. La moyenne d'âge des sujets présentant une infection concomitante à E. histolytica, A. lombricoïdes et Plasmodium (7.55±2.03 ans) était significativement bas par rapport à celle des sujets avec infection concomitante à E. histolitica. A. lombricoïdes mais sans parasites palustres (17.35±14.89 ans): t=4.21, P<0.05. L'infection concomitante à E. histolytica et A. lombricoïdes diminuerait donc l'immunité au paludisme chez les enfants. Les sujets infectés par P. falciparum avaient un hématocrite significativement plus bas (36.72±4.92%) que celui observé chez les sujets sans P falciparum (40.16±4.58%): t=4.21, P<0.05. Pareillement, les sujets avec N. americanus avaient un hématocrite (37.9±5.08%) significativement plus bas que ceux n'en ayant pas (40.00±4.82%) : t=2.48, P<0.05. Conséquemment, en régions endémiques à infection concomitante au paludisme et infection intestinales, les infections simultanées au P. falciparum et au N. americanus contribueraient dans l'anémie.

Mots clés: paludisme, parasites intestinaux, infections concomitantes, immunité

INTRODUCTION

Intestinal parasitoses have been neglected because their direct impact on human life has not been well defined. However, that attitude has been rejected since intestinal infections affect more than two thirds of the human population and mostly children¹⁰. Ascaris lumbricoides, Necator americanus and amoeba are among the major intestinal parasites that affect humans. Helminths affect 70% of the world's population¹². They are closely linked to faecal peril and, more especially, prevalent amongst populations in developing countries. 12

Epidemiological surveys to evaluate the frequency of the various intestinal helminths are usually difficult to compare because of the different coprological techniques used. Their prevalence depends on several physical, environmental and socio-economic factors^{10,11} such as poverty, insanity as well as the lack of proper hygiene and potable water. In developing countries, sewage disposal constitutes a major source of contamination. Sometimes, fresh stools are used as fertilizers and may contain viable eggs and larvae. ¹⁰

Where fishing and farming are the main economic pursuit of the community, intestinal parasitoses occur without age discrimination¹. Heavily infected people play an important role in the perennial dissemination of the parasites and patients often show signs of morbidity that may be amplified by multiple concomitant intestinal infections. Such multiple infection is sometimes

associated with malaria. Little is known about the effects of concomitant intestinal parasitosis on malaria infection and immunity. Nevertheless, it has been reported that intestinal worms contribute to the down regulation of the immune response to malaria.⁸

This study is intended to contribute to a better understanding of the relationship between multiple and concomitant intestinal parasitic infections in Simbok, a malaria endemic village in the forested area of Cameroon.

MATERIALS AND METHODS

Permission and consent:

Permission to undertake field studies in the village was authorized by the Ministry of Health. After reaching agreement with the authorities of the village, details of field and laboratory procedures were explained. Participation in this project was voluntary.

Study site and study population:

This study was conducted from November 1996 to May 1997 in Simbok, a malaria endemic village located on the outskirts of Yaounde, the capital city of Cameroon. Census conducted in 1996 showed that 907 people live in Simbok in 160 households. The number of residents per household ranged from 2 to 20 persons. This village is located 11°27°N, 3°49°W within the rainforest belt of Central Africa (Fig. 1). The residents of Simbok are farmers and most of the children

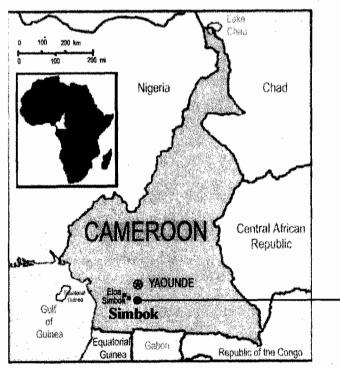


Figure 1: The map of Cameroon localising Simbok village

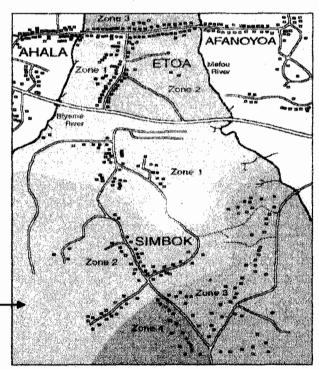


Figure 2: The map of Simbok village showing households, roads and hydrography

are students. The region has a wet tropical climate, with temperature ranging from 17°C to 30°C (mean = 23.1°C)^{5, 9, 15}. The mean annual rainfall averages between 1.587mm and 1.700mm^{9, 15}, with the average relative humidity index ranging from 85% to 90%. The two surrounding rivers [Biyeme river and Mefou river (Fig. 2)] irrigate the farms and also form swamps.

Because the village is large and spread out, it was divided into three zones. A village helper was assigned to each zone. Central location was identified for each zone where villagers assembled in the morning for the survey. An identification number was assigned to each subject and the following parameters recorded: age, sex, axillary temperature and body weight. Fever and chill were defined as axillary temperature +0.5 > 37.5°C and axillary temperature + 0.5 ≤ 36.5 °C respectively. Temperature within the range of 36.5 - 37.5°C was considered as normal¹⁸ Thin and thick smears were made from finger-pricked blood for malariology. Peripheral blood collected in EDTA tubes was used for haemoglobin genotyping by electrophoresis, for ABO blood grouping and for packed cell volume (PCV) determination using a micro centrifuge. The PCV was used to evaluate the level of anaemia. Anaemia and severe anaemia were defined as PCV<32% and PCV≤22% respectively ^{19, 20}. About 4 cm³ stool sample was collected for intestinal parasitology.

Laboratory tests

Thin and thick blood films were made on the same slide, air dried at room temperature (RT), stained using Field stain and examined for the presence of malaria parasites by microscopy. Each slide was read and confirmed by two different microscopists. The presence of malaria parasites in the blood was considered as confirmation of diagnosis while a blood smear was considered negative only after 200 oil immersion fields of thick smear have been examined

From each subject, about 4 cm³ fresh stool were collected in a plastic tube and taken to the laboratory for analysis. Samples were processed according to WHO 1980¹⁷. One drop of sodium chloride solution and one drop of iodine solution were separately put on a slide. Using an applicator, a portion from well inside the formed sample and from the surface was mixed with the drop of sodium chloride solution on the slide. For stools that

Table 1: The prevalence of malaria and intestinal parasites taken individually

malaria	Entamoeba histolytica	Necator americanus	Ascaris lumbricoides	Ancylostoma	Schistosoma mansoni	Trichomonas	Yeast	Giardia	Isospora	E. coli
23.9%	43%	23.2%	23.2%	8.6%	7.6%	1.6%	7%	3.2%	0.5%	2.2
(43/180)	(80/185)	(43/185)	(43/185)	(16/185)	(14/185)	(3/185)	(13/185)	(6/185)	(1/185)	(4/185)

Table 2: The prevalence of concomitant 2 infections

	Malaria	Entamoeba histolytica	Ascaris lumbricoides
Necator americanus	6.1% (11/180)	11.35% (21/185)	12.77% (23/185)
Ascaris lumbricoides	9.2% (17/180)	12.97% (24/185)	
Entamoeba histolytica	12.77% (23/180)		

Table 3: The prevalence of concomitant infections with 3 species of parasites

A	В	С	D	E	F	G	Н	I	J	K	L	M
7.0%	0.5%	5.4%	0.5%	5.4%	1.1%	1.1%	1.1%	1.1%	0.5%	0.5%	1.1%	0.5%
(13/185)	(1/185)	(10/185)	(1/185)	(10/185)	(2/185)	(2/185)	(2/185)	(2/185)	(1/185)	(1/185)	(2/185)	(1/185)

Legend

- A = Entamoeba histolytica + Necator americanus + Ascaris lumbricoides
- B = Malaria+ Necator americanus + Schistosoma mansoni
- C =Malaria+ Necator americanus + Ascaris lumbricoides
- D = Entamoeba histolytica + Schistosoma mansoni + Trichomonas
- E = Malaria+ Entamoeba histolytica + Ascaris lumbricoides
- F = Malaria + Entamoeba histolytica + Giardia
- G = Entamoeba histolytica + Ascaris lumbricoides + E. coli
- H = Malaria + Entamoeba histolytica + E. coli
- I = Necator americanus + Ascaris lumbricoides + Yeast
- J = Necator americanus + Ascaris lumbricoidess + Isospora
- K = Malaria+ Ascaris lumbricoides + Giardia
- L = Malaria + Ascaris lumbricoides + Ancylostoma
- M = Malaria + Entamoeba histolytica + Yeast

Table 4: The prevalence of concomitant infections with 4 species of parasites

Species	N	0	P	Q	R	S	T
Prevalence	1.6%	1.1%	3.8%	1.1%	0.5%	1.1%	0.5%
<u> </u>	(3/185)	(2/185)	(7/185)	(2/185)	(1/185)	(2/185)	(1/185)

Legend

- N = Entamoeba histolytica + Necator americanus + Ancylostoma + Schistosoma mansoni
- **O = Entamoeba histolytica** + Necator americamus + Ascaris lumbricoides + Ancylostoma
- P = Malaria+ Entamoeba histolytica + Necator americanus + Ascaris lumbricoides
- Q = Malaria + Necator americanus + Ascaris lumbricoides + Ancylostoma
- R = Entamoeba histolytica + Necator americanus + Ascaris lumbricoides + Yeast
- S = Entamoeba histolytica + Necator americanus + Ascaris lumbricoides + Schistosoma mansoni
- T = Malaria + Entamoeba histolytica + Necator americanus + E. coli

contained mucus or were liquid, a portion was taken from the bloodstained mucus on the surface or from the surface of the liquid. A second portion of stool from the specimen was mixed with the drop of the iodine solution. A coverslip was placed over each drop and the preparations examined under a light microscope equipped with a condenser (X40 objective for iodine solution preparation; X10 and X40 objectives and X5 eyepiece for the saline preparation).

Case management:

Patients were freely treated following the medical doctor's prescription as follows: albendazol for helminths and metronidazol for *Amoeba* were given for intestinal parasites and chloroquine was given for malaria. Paracetamol and iron tablets were donated as analgesic drug and blood supplement respectively. "ENO", which is a digestion facilitating salt, was given to negative subjects as placebo.

Statistical analysis

 χ^2 test was used to compare prevalence rates and z test and Students t-test to estimate the differences between the mean values at a 95% confidence interval.

Result

Of the 185 volunteers who took part in the study, 58.4% (108/185) were female and 41.6% (77/185) were male; 25.4% (47/185) aged between 5 and 10 years while

20% (38/185), 49.7% (92/185) and 2.7% (5/185) aged between 10 and 15 years, 16 and 65 years, and above 65 years respectively. 20.5% (38/185), 2.7% (5/185), 22.1% (41/185) and 54.5% (100/185) were blood group A, B, AB and O respectively. 70.8% (131/185) and 26.5% (49/185) subjects were AA and AS genotype respectively. 6.5% (12/185) of the subjects were anaemic (PCV<32%) while 93.5% (173/185) were not. 71.9% (133/185) had normal temperature (36.5 – 37.5°C) while 14.6% (27/185) and 13.5% (25/185) had low temperature and fever respectively.

Our results showed that malaria and intestinal parasites are prevalent in Simbok. The prevalence of malaria was 23.9% (43/180). Entamoeba histolytica was the most prevalent [43% (80/185)] intestinal parasite in the studied subjects (Table 1). The overall prevalence of intestinal parasites was 68.6% (127/185) while that of helminths alone was 43.2% (80/185). The prevalence of various intestinal parasites was reported in Table 1.

Concomitant infections involving 2 to 4 infections were recorded in the studied population (Table 2-4).

The prevalence of most parasitic infections decreases with age, amoeba, *Ascaris lumbricoides* and malaria being the most prevalent. The comparison of the prevalence of the studied infections between children and adults are recorded in table 5.

Table 5: The prevalence of malaria and intestinal parasites in different age groups.

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Age group (Years)	Malaria	Entamoeba histolytica	Necator americanus	Ascaris hanbricoides	Ancylostoma	Schistosom a mansoni	Trichomonas	Yeast	Giardia	Isospora	E. coli
< 10	47.2% (17/36)	61.1% (22/36)	41.7% (15/36)	52.8% (19/36)	8.3% (3/36)	5.6% (2/36)	0% (0/36)	13.9% (5/36)	11.1% (4/36)	2.8% (1/36)	0% (0/36)
[10 - 15]	32% (16/50)	56% (28/50)	34% (17/50)	26% (13/50)	10% (5/50)	16% (8/50)	2% (1/50)	10% (5/10)	4% (2/50)	0% (0/50)	0% (0/50)
> 15	10.6% (10/94)	35.4% (35/99)	13.1% (13/99)	13.1% (13/99)	8.1% (8/99)	4% (4/99)	2% (2/99)	3% (3/99)	0% (0/99)	(0/99)	4% (4/99)
≤15	37.5% (33/86)	58.1% (50/86)	37.2% (32/86)	37.2% (32/86)	9.3% (8/86)	11.6% (10/86)	1.2% (1/86)	11.6% (6/86)	7% (6/86)	1.2% (1/86)	0% (0/86)
	<0.001	<0.001	<0.001	<0.001	>0.5	<0.001	>0.5	<0.001	-	-	0.05
				Pysh	es (hetwee	n adults an	d non adult	(2			

Figure 3 illustrates the relationship between the prevalence of infections with malaria parasites, *Entamoeba histolytica*, the overall helminths and age.

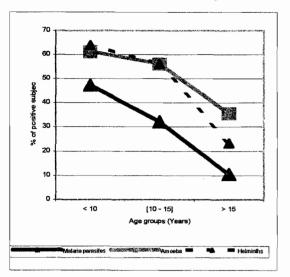


Fig. 3: Relationship between the prevalence of infections with malaria parasites, *Entamoeba histolytica*, helminths and age

Single infections with intestinal parasite were more frequent than concomitant intestinal parasite and malaria infections (Fig. 4). Interestingly, the mean age of subjects with concomitant *Entamoeba histolytica*, *Ascaris lumbricoides* and malaria infections (7.55 \pm 2.03) significantly differed from that of those who presented with concomitant *Ascaris lumbricoides* and *Entamoeba histolytica* without malaria infection (17.35 \pm 14.89). (t = 2.0; P = 0.05) (Fig. 5).

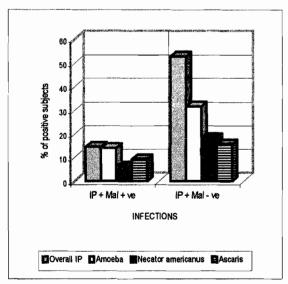


Fig. 4: The prevalence of concomitant malaria and intestinal parasites

Legend I.P.:

I.P.: Intestinal parasites
As: Ascaris lumbricoides
Eh: Entamoeba histolytica

MAL: Malaria
-ve: Negative
+ve: Positive

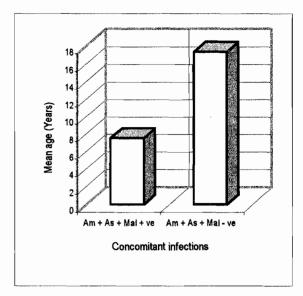


Fig. 5: Relationship between concomitant malaria, intestinal infections and age.

Subjects with malaria parasites had low PCV (36.72 \pm 4.92%) compared to those without malaria parasites (40.16 \pm 4.58%) (t = 4.21; P < 0.05) (Fig. 6). Similarly, subjects with *Necator americanus* had low PCV (37.9 \pm 5.08 %) compared to those without *Necator americanus* infection (40.00 \pm 4.82 %). (t = 2.48; P<0.05) (Fig. 7).

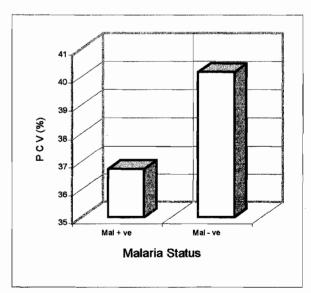


Fig. 6: Relationship between malaria infection and PCV level

DISCUSSION

The prevalence of malaria in the studied subjects was seemingly low (23.9%), compared to the results obtained from a three year prevalence study conducted in

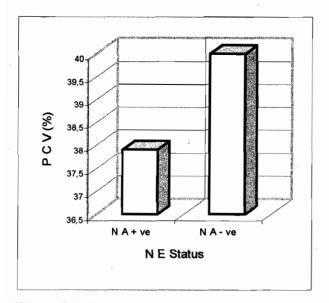


Fig. 7: Relationship between *Necator* americanus infection (NA) and PCV level NA = Necator americanus

the same village, but in the whole population without excluding babies and infants (1996:41%; 1997:45%; 1998: 55%.)¹³. Among the studied subjects, malaria was most prevalent in children (5 – 10 years old). This observation was similar to the results obtained in most of the malaria endemic areas. ^{3, 6, 7, 13}. From this observation, it is assumed that young people may be more susceptible to malaria and that they build resistance to malaria with age. In fact, it is well documented that in areas with endemic and perennial malaria, once the immunity to malaria passively acquired from the mother goes down, infants take a long time to build up their own protective immunity as they grow up¹³.

Similarly, the prevalence of most of the intestinal parasites decreased with age. This may be due to several reasons. First, old children and adults are more conscious of hygiene and sanitation than young children. Secondly, young children are more infected and probably more active in maintaining transmission. In addition, schools in the study area have overcrowded classrooms, no or unhygienic latrines and no hygienic practices after defaecation. Another factor is the defaecation behaviour of children, which is likely to be different from that of adults. Most studies conducted in this domaine have reported high prevalence and heavy intensities of intestinal parasitic infections in children. In the study of the studies of intestinal parasitic infections in children.

The high prevalence of the overall intestinal parasite in the village may be due to several factors. First, lack of potable water in Simbok. The indigenes rely on water from doubtful sources (streams...). Secondly, the principal activity of the village population is farming and they still rely on primitive methods. By so doing, they are usually in direct contact with infected earth, which enhance their contamination.

The overall prevalence of intestinal helminths in the studied subjects was 43.2%. The result may reflect an underestimation in Simbok because the method we used might not have been accurate enough to detect scanty parasitaemias. Entamoeba histolytica, Necator americanus, Ascaris lumbricoides, Yeast, E. coli, Giardia, schistosoma mansoni, Trichomonas, Isospora, and Ancylostoma were encountered at various rates. Further analysis combined with coproculture should demonstrate many more intestinal parasite species in Simbok. Most of the parasites identified were potentially pathogenic but the infection level in the cases examined was not high enough for the infections to be symptomatic. Results on the prevalence of Acylostoma duodenal are not surprising since previous studies by Gentilini21 and Ranque22; and their respective collaborators have demonstrated that it is highly endemic in all countries with hot and humid climate.

Contrary to the observed high prevalence and high intensities in adult population in Kenya^{4, 10}, it was observed that adults in Simbok had a low prevalence of *Schistosoma mansoni* compared with children (P<0.001)

The mean age of subjects with concomitant *Entamoeba histolytica*, *Ascaris lumbricoides* and malaria infections was significantly lower than those with concomitant *Ascaris lumbricoides* and *Entamoeba histolytica* but without malaria infection (P = 0.05). Therefore, concomitant infections with *Ascaris lumbricoides* and *Entamoeba histolytica* may downregulate immunity to malaria in children. Then, young persons may be more vulnerable to concomitant *Ascaris lumbricoides* and *Entamoeba histolytica* infections associated to malaria. Other factors like MHC type and behavioural factors are suspected to play a role in the susceptibility to concomitant malaria and intestinal parasite infections¹⁰.

CONCLUSION

Simbok is endemic for malaria and concomitant intestinal parasites endemic area. Those infections affect people of all the age groups. People with either Malaria or *Necator americanus* infections; or with both infections are more likely to be anaemic. Many subjects harboured parasites without developing the disease. In order to prevent dissemination of the parasites, it may be better to systematically treat all the parasite carriers. Treatment only may not be sufficient for the disease control strategy at the community level. Under conditions like in the present study area, effective control of at least the most prevalent infections (malaria,

amoebiasis and helminthiasis) requires preventive methods (impregnated bed net and drugs) as well as good hygiene and sanitation measures.

It would be better to use serological and molecular biology methods to have a better understanding of the malaria and intestinal infection pathway as well as the immune response they induce. The study of genetic factors may also enable us to have a clear knowledge of the susceptibility to malaria and concomitant intestinal parasites.

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- The Biotechnology Centre of the University of Yaounde 1 where most of the laboratory work was carried out.

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