Co-infections of Malaria and Geohelminthiasis in Two Rural Communities of Nkassomo and Vian in the Mfou Health District, Cameroon

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Introduction

In many afro-tropical countries, parasitic co-existence is common with increased potential for co-infection, which may adversely impact the outcome of the diseases they cause. [1]. Of all human diseases caused by protozoan parasites malaria has the greatest burden and is responsible for most deaths amongst young children in sub-Saharan Africa, accounting for 90% of all global cases [2]. Until the past decade, intestinal worms have been neglected due to insufficient knowledge of their impact on human life [3–6]. The fact that intestinal worms affect more than two-thirds (70%) of humans has led to growing interest to understand their epidemiology and interactions with other parasitic infections [7,8]. In Cameroon, both malaria and helminth infections co-exist and are ranked amongst the major cause of parasitic mortality and morbidity. Plasmodium falciparum is the most prevalent and virulent of the malaria parasites [9–12], while the geohelminths (Ascaris lumbricoides, Trichuris trichiura, and Hookworm spp.) and Schistosoma mansoni are the major helminthic parasites [3,13–16]. Although there is much literature on the epidemiology

Abstract

Background: Human co-infection with malaria and helminths is ubiquitous throughout Africa. Nevertheless, its public health significance on malaria severity remains poorly understood.

Methodology/Principal Findings: To contribute to a better understanding of epidemiology and control of this co-infection in Cameroon, a cross-sectional study was carried out to assess the prevalence of concomitant intestinal geohelminthiasis and malaria, and to evaluate its association with malaria and anaemia in Nkassomo and Vian. Finger prick blood specimens from a total of 263 participants aged 1–95 years were collected for malaria microscopy, assessment of haemoglobin levels, and molecular identification of Plasmodium species by PCR. Fresh stool specimens were also collected for the identification and quantification of geohelminths by the Kato-Katz method. The prevalence of malaria, geohelminths, and co-infections were 77.2%, 28.6%, and 22.1%, respectively. Plasmodium falciparum was the only malaria parasite species identified with mean parasite density of 111 (40; 18,800) parasites/μl of blood. The geohelminths found were Ascaris lumbricoides (21.6%) and Trichuris trichiura (10.8%), with mean parasite densities of 243 (24; 3,552) and 36 (24; 96) eggs/gram of faeces, respectively. Co-infections of A. lumbricoides and P. falciparum were the most frequent and correlated positively. While no significant difference was observed on the prevalence of single and co-infections between the two localities, there was a significant difference in the density of A. lumbricoides infection between the two localities. The overall prevalence of anaemia was 42%, with individuals co-infected with T. trichiura and P. falciparum (60%) being the most at risk. While the prevalence of malaria and anaemia were inversely related to age, children aged 5–14 years were more susceptible to geohelminthiasis and their co-infections with malaria.

Conclusion/Significance: Co-existence of geohelminths and malaria parasites in Nkassomo and Vian enhances the occurrence of co-infections, and consequently, increases the risk for anaemia.

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Introduction

In many afro-tropical countries, parasitic co-existence is common with increased potential for co-infection, which may adversely impact the outcome of the diseases they cause. [1]. Of all human diseases caused by protozoan parasites malaria has the greatest burden and is responsible for most deaths amongst young children in sub-Saharan Africa, accounting for 90% of all global cases [2]. Until the past decade, intestinal worms have been neglected due to insufficient knowledge of their impact on human life [3–6]. The fact that intestinal worms affect more than two-thirds (70%) of humans has led to growing interest to understand their epidemiology and interactions with other parasitic infections [7,8]. In Cameroon, both malaria and helminth infections co-exist and are ranked amongst the major cause of parasitic mortality and morbidity. Plasmodium falciparum is the most prevalent and virulent of the malaria parasites [9–12], while the geohelminths (Ascaris lumbricoides, Trichuris trichiura, and Hookworm spp.) and Schistosoma mansoni are the major helminthic parasites [3,13–16]. Although there is much literature on the epidemiology
Co-infections of malaria and intestinal helminths causes significant and additive problems against the host. In order to contribute to a better understanding of the epidemiology and control of concomitant intestinal geo-helminthiasis and malaria infections in Cameroon, a cross-sectional study to assess its prevalence and evaluate its influence on malaria and anaemia was carried out in two rural communities (Nkassomo and Vian) of the Mfou health district. Fresh stool and finger prick blood samples from 263 participants aged 1–95 years were analysed to identify and quantify the geo-helminths and malaria parasites respectively. Anaemia was assessed by measuring the haemoglobin levels. Whereas *Plasmodium falciparum* (77.2%) was the only malaria parasite species found in the study population, *Ascaris lumbricoides* (21.6%) and *Trichuris trichiura* (10.8%) were the only geo-helminths detected. The prevalence of malaria and geohelminth co-infections was 22.1% with co-infections of *A. lumbricoides* and *P. falciparum* being the most frequently encountered and associated. The prevalence of anaemia was 42%, with the most affected being those with co-infections of *T. trichiura* and *P. falciparum*. Schoolchildren had significantly higher risk of geohelminthiasis, and their coinfection is a cause for concern and should strategically be considered in the designing and implementation of their control.

of malaria and intestinal worms separately, little is known about the distribution and impact of their co-infections on the population across the country [11,17]. Due to the differences in the physiological, anthropological, genetic, immunological or geo-ecological factors, infections with multiple parasite species may not necessarily be independent within an individual, and could result in positive or negative associations in disease manifestation. The implications of concomitant malaria and helminth infections have been mainly explored and indicate that their interactions can decrease the course of malaria infection and disease [18,19]. On the other hand, co-infected school-aged children have been shown with different phenotypes in the pathogenesis of malaria with increasing risk of developing severe malaria [1,10,18,20–22]. These conditions could lead to increased malaria parasitaemia, increased risks of anaemia and malnutrition, modification of the immune response to malaria [12,23,24], and increased mortality. Therefore, the acquisition of requisite information on co-morbidity and interactions between malaria and helminthiasis would be invaluable to control malaria infection and clinical disease. The control of these parasitic diseases in Cameroon rely on the WHO strategies, which lay emphasis on a combined control approach for malaria infection [11] and deworming activities for helmith parasites [17]. However, implementation of such measures requires essential requisite information on the geo-ecological distribution and efficiency in infection transmission. Thus, this study sought to determine the prevalence of malaria and geohelminths co-infection and evaluate its impact on malaria and anaemia in Nkassomo and Vian, two rural communities of Cameroon. The findings from the study will provide useful information necessary to design strategies to effectively control and manage malaria in the context of co-infection.

**Materials and Methods**

**Ethical considerations**

This study received ethical and administrative authorizations respectively from the IMPM institutional ethics review committee and the competent authorities of the Mfou health district. Participation was strictly voluntary and was dependent on informed written consent by the participant or assent by the parent/guardian for children. All positive cases for malaria and/or intestinal worms were treated for free according to the Cameroonian anti-malarial and anti-helminth treatment guidelines and if necessary referred to the Mfou district hospital for appropriate management. A single dose of Paracetamol and iron tablets was respectively given to participants with fever or anaemia.

**Study area**

The study was carried out in two rural communities (Nkassomo and Vian) of the Mfou health district (4°27’N and 11°38’E), a forest area located in the Mfou-Afamba division, in the Centre region of Cameroon. The climate is typically equatorial with two discontinuous dry and wet seasons. The annual average rainfall measures 2,000 mm with an annual average temperature of 24 °C. The population is made up of 71,373 inhabitants (4,100 in Nkassomo and 3,248 in Vian) with about 82.7 inhabitants/km². Mfou is a multiethnic community made up of the Ewondo, Bané, and Tsinga, with farming being the main economic pursuit.

**Study population**

The study was conducted from February to March 2011 during the long dry season in the Mfou health district. Samples were collected from each household and only from individuals who willingly accepted to participate and provided written and signed informed consent and have lived in the village for at least the past six months. A total of 263 participants from the two rural communities (131 from Nkassomo and 132 from Vian) aged 1 to 95 years old took part in the survey and were divided into 6 groups as follows: pre-school children (<5 yrs), young school children (5–9 yrs), old school children (10–14 yrs), adolescents and young adults (15–24 yrs), adults (25–49 yrs), and aged people (≥50 yrs). Each participant was clinically examined by a medical doctor for febrile symptoms (fever, axial T ≥37.5°C) or any other clinical conditions (headache, abdominal discomforts, etc.), and information on age and sex taken prior to specimen collection.

**Sample collection and processing**

**Blood collection and processing.** Finger prick blood samples collected in heparinised micro-capillary tubes were used to prepare thick and thin smears for malaria microscopy, blot filter papers (Whatmann #3) for *Plasmodium* DNA isolation and PCR amplification for species determination, and measure the haemoglobin level.

**Detection and quantification of malaria parasites.** The thin and thick blood smears air-dried were fixed in 75% methanol and 50% May-Grunwald (thick film only) and stained using 10% Giemsa solution for both thin and thick films. Slides were then microscopically examined for the presence of malaria parasites by two independent microscopists. Where parasites were found the parasite density was determined by counting the number of parasites against 200 leucocytes. The counts were expressed as the number of parasites per micro litre of blood (p/μl), assuming an average leukocyte count of 8,000 cells/μl of blood [26]. For every slide where the difference in parasitaemia between the two readers...
was greater than 5 p/µl of blood, a third reader had to re-examine the slide and the mean of the two closest values considered.

**Plasmodium DNA extraction and amplification.** Fresh blood specimens from heparinised micro-hematocrit tubes were transferred onto Whatmann #3 filter paper and used for the extraction of Plasmodium DNA and subsequent PCR amplification for the identification of Plasmodium species. [27–29]

**Assessment of anaemia.** A drop of capillary blood was set down on a strip of a portable haemoglobinometer (URIT MEDICAL ELECTRONIC Co, LTD, 2008; URIT-12; <58 g;
102 mm×50 mm×19 mm; DC 3 V; 8 years) to determine the total haemoglobin level according to the product instructions. Anaemia was considered as total haemoglobin level <11 g/dl of blood and classified according to the WHO definition as either mild, moderate or severe [30].

**Stool collection and processing.** Prior to stool sampling, clear instructions and clean well labelled stool collection vials were given to each participant for proper stool collection. Stool smears were prepared using the Kato Katz technique for the morphological identification of geohelminth eggs for _A. lumbricoides, T. trichiura_, and Hookworms _sp_., or larval stage for _S. stercoralis_ [31]. The smears were made on duplicate slides per participant, kept refrigerated overnight and then microscopically examined within 7–9 hrs to avoid missing hookworm eggs and minimize bias on the parasitological data. Helminth eggs were counted in 41.7 mg of stool and counts were extrapolated as the number of eggs per gram of stool (eps).

**Statistical analysis**

Statistical analysis of the data was performed using SPSS 18.0 software (SPSS Inc, Chicago IL, USA). Chi-square test and One Way ANOVA were used to set the difference in proportions and means respectively, whereas the Pearson logistic regression test was used to establish the correlation between variables (Plasmodium densities, geohelminth densities, fever, and anaemia). Threshold for statistical significance was set at $P<0.05$. Prevalence was defined as the proportion of individuals found harbouring the parasite in question to the sum total of the study population. Co-infection was defined as the simultaneous presence of at least one helminth parasite species plus malaria parasite in the same host, and was classified as either single or mixed, depending on the number of parasites found. Qualitative values were expressed in frequency or percentage, whilst quantitative values were expressed as geometric mean values (minimum value; maximum value) or arithmetic mean value ± Standard Deviation (SD).

**Results**

**Demography of the study population**

Of the 263 study participants 131 (49.8%) were from Nkassomo and 132 (50.2%) from Vian. Of these, there were 125 (49.6%) males and 139 (53.1%) females. The median age in the study population was 26.1±22.4 yrs (range, 1 to 95 yrs). Based on the WHO criteria, the median axial temperature was 37.3±0.5°C (range, 35.5 to 41°C) and 91 (34.6%) participants had fever. Although children in the age group of 5–9 yrs had the most cases of fever no difference was observed based on age, sex and locality. A total of two hundred and eighteen participants (82.9%) was found to carry at least one of the identified parasites (Plasmodium falciparum, _A. lumbricoides_ or _T. trichiura_).

**Parasite prevalence and intensities**

The overall prevalence of malaria was 77.2% (n = 203) with _Plasmodium falciparum_ being the only parasite species found. The mean parasite density in the population was 111 (40; 18,800) parasites per micro litre of blood (P/µl). Based on the study locality, the prevalence of malaria in Nkassomo was 76.8% (n = 103) and 75.8% (n = 100) in Vian. This did not vary significantly in the prevalence. A similar trend was observed for the parasite density with an average parasite load of 125 (40; 14,760) P/µl in Nkassomo, and 90 (40; 10,800) P/µl in Vian. Sex did not influence the prevalence and intensity of _P. falciparum_ in both localities. Although all age groups were affected, the peak of malaria prevalence was observed in children less than 9 years old. Only 32.5% (n = 66) of malaria positive cases had fever and with the highest parasite densities (Tables 1 and 2). No gametocytes were found in the infected persons.

_Acaris lumbricoideis, Trichurus trichiura_ were the only geohelminth parasites detected. Of the 66 (28.6%) participants positive for geohelminth infection, there were 50 (21.6%) _A. lumbricoideis_, 25 (10.8%) _T. trichiura_. However, there were 9 (3.9%) cases with both infections. The geometric mean of the parasite density for each species was 243 (24; 3,552) eggs per gram of faeces (eps) for _A. lumbricoideis_, and 36 (24, 96) eps for _T. trichiura_. There was no observed significant difference in the prevalence of the two parasites between the two localities. However, the parasite densities were higher in Vian with a significant difference observed for _A. lumbricoideis_ (p = 0.001). With regards to sex and age, the prevalence of _A. lumbricoideis_ was similarly distributed and was higher in children between the ages of 5 years and 14 years (p = 0.002), whilst no difference was observed for _T. trichiura_ infection between the males and the females (p = 0.071) and by age groups. Overall, school-aged children (5–14 yrs) had the highest prevalence and parasite density of geohelminth infections. However, only the prevalence was significantly different when compared to other age groups (p = 0.008) (Table 1).

With regards to malaria and geohelminth co-infection, a total of 51 (22.1%) participants were infected with at least one geohelminth parasite and _P. falciparum_. Two cases of co-infections were observed and classified as either single co-infections of _A. lumbricoideis_ and _P. falciparum_ (38 [16.5%]), and of _T. trichiura_ and _P. falciparum_ (20 [8.7%]); or mixed co-infections of the three parasites (7 [3.0%]). While observed with single infections of individual parasites, there was significant difference in the prevalence of co-infections between the two study localities. Co-infections with _T. trichiura_ and _P. falciparum_ was observably higher in males than females (p = 0.009). Similarly the overall co-infection was also higher in males than females (p = 0.025). The highest prevalence of co-infection was observed in children between 5 and 14 years old (p = 0.010). With regards to the parasite densities, single infections with only _A. lumbricoideis_ (179 [24; 1,920] eps) and only _P. falciparum_ (107 [40; 4,000]) p/µl had generally lower parasite densities compared to when the two occurred as co-infections of _A. lumbricoideis_ (257 [24; 3,552] eps, p = 0.012) and _P. falciparum_ (143 [40; 2,360] p/µl, p = 0.018). Although there was a similar observation for mixed co-infections for _A. lumbricoideis_, _T. trichiura_ was associated with low densities of _P. falciparum_, but the difference was not significant (Table 3). On the other hand, logistic regression analysis (Figure 1) showed a positive association between the parasite densities of _A. lumbricoideis_ (independent variable) and _P. falciparum_ (dependant variable) in co-infected individuals (R = 0.406, Correlation coefficient = 0.381, p = 0.175).

**Anaemia and infections**

According to the WHO classification, out of the 263 participants in the study, 110 had haemoglobin levels less than 11 g/dl of blood, giving an overall anaemia prevalence of 42%. However,
Table 1. Baseline data of the study population and distribution of fever, *Plasmodium spp*, geohelminth *spp*, co-infections, and anaemia by locality, sex, and age group.

| Locality | Sex | Age (yrs) | Total/Mean | p-value |
|----------|-----|-----------|------------|---------|
|          |     | <5        | 5–9        | 10–14   | 15–24   | 25–49   | >50     |         |
|          |     |           |            |          |          |          |          |         |
| NKVI     | M   | 131(49.8) | 132(50.2)  | 123(49.6) | 139(53.1)| 32(12.5) | 49(19.1)| 39(15.2)| 32(12.5) | 49(19.1)| 55(21.5)| 263(100) | -        | 0.265   | 0.278   |
|          | F   | 41(31.3)  | 50(37.9)   | 47(38.2)  | 44(31.7) | 14(33.3) | 24(49.0)| 15(38.5)| 11(34.4)| 14(28.6)| 12(21.8)| 91(34.6) | 0.262   | 0.266   | 0.006   |
| P.f      |     | 103(78.6)| 100(75.8)  | 92(74.8)  | 111(79.9)| 27(84.4)| 43(87.8)| 28(71.8)| 26(81.2)| 35(71.4)| 39(70.9)| 203(77.2)| 0.579   | 0.328   | 0.208   |
| A.l      |     | 24(19.2)  | 26(24.5)   | 24(22.2)  | 25(20.5) | 4(13.3)  | 12(27.3)| 17(45.9)| 6(13.0) | 16(33)  | 50(21.6) | 0.312   | 0.749   | 0.002   |
| T.t      |     | 34(24.9)  | 37(24.7)   | 16(14.8)  | 9(7.4)   | 2(7.1)   | 5(11.4) | 6(16.2) | 4(14.8) | 5(10.9) | 24(44)   | 0.022   | 0.071   | 0.560   |
| A.l-T.t  |     | 3(2.4)    | 6(5.6)     | 3(2.4)    | -        | 2(4.5)   | 3(8.1)  | 1(3.7)  | 12(2.2) | 12.2    | 9(3.9)   | 0.061   | 0.878   | 0.261   |
| A.l-P.f  |     | 18(14.4)  | 20(18.9)   | 20(18.5)  | 18(14.8) | 4(14.3)  | 12(27.3)| 12(24.4)| 4(14.8) | 48(76)  | 24(44)   | 0.361   | 0.097   | 0.034   |
| T.t-P.f  |     | 11(8.8)   | 9(7.8)     | 15(13.9)  | 5(4.1)   | 2(7.1)   | 5(11.4) | 3(8.1)  | 4(14.8) | 48(76)  | 122(24)  | 0.034   | 0.097   | 0.034   |
| A.l-T.t-P.f|   | 21.6     | 5(4.4)     | 4(3.6)    | 3(2.4)   | -        | 2(4.5)  | 3(8.1)  | 1(3.7)  | -       | 12(2.2) | 7(3.0)  | 0.194   | 0.602   | 0.294   |
| Hb^a     |     | 12.7±1.3  | 12.7±1.5   | 13.0±1.6  | 12.4±1.1 | 12±1.0   | 11.9±0.8| 11.9±0.7| 12.9±1.1| 13.5±1.7| 12.9±1.3| 12.7±1.4| 0.420   | 0.524   | 0.358   |
| Hb^b     |     | 9.8±0.8   | 9.7±0.9    | 9.8±0.9   | 9.8±0.9  | 9.5±1.0  | 9.7±0.8 | 10±0.7 | 9.8±0.9 | 10±0.8  | 10.2±0.7| 9.8±0.8 | 0.261   | 0.815   | 0.295   |
| A         |     | 53(40.5)  | 57(43.5)   | 47(39.8)  | 63(43.9) | 27(48.4)| 26(53.1)| 17(43.6)| 9(28.1)| 14(28.6) | 11(20)   | 110(42) | 0.167   | 0.508   | 0.003   |
| MPD       |     | 125(40;14750)| 98(40;18800)| 121(40;40000)| 103(40;18800)| 170(40;18800)| 163(40;2360)| 95(40;801)| 96(40;1840)| 73(40;1080)| 86(40;1320)| 111(40;18800)| 0.161   | 0.398   | 0.081   |
| MAD       |     | 94(24;2088)| 58(24;3552)| 262(24;3552)| 231(24;2352)| 87(24;2088)| 250(24;2400)| 448(24;3552)| 93(24;1344)| 247(24;2352)| 164(24;1776)| 243(24;3552)| 0.001   | 0.448   | 0.587   |
| MTD       |     | 34(24.96)| 37(24.72)  | 36(24.96)| 34(24.72)| 42(24.72)| 48(24.96)| 29(24.72)| 40(24.48)| 36(24.48)| 24(24.72)| 36(24.96)| 0.687   | 0.705   | 0.463   |

Proportions are expressed as number of cases with percentages in brackets, arithmetic values are expressed as mean ± Standard Deviation (SD), and geometric values are expressed as mean value and maximum values in brackets. NK: Nkassomo, VI: Vian, M: male, F: female, N: number of participants, MAT: arithmetic mean axillary temperature in degree Celsius, Fever cases, P.f: Plasmodium falciparum, A.I: Ascaris lumbricoides, T.t: Trichuris trichiura, A.I-T.t: polyparasitism of Ascaris and Trichuris, A.I-P.f: single co-infection of Ascaris and Plasmodium, T.t-P.f: single co-infection of Trichuris and Plasmodium, A.I-T.t-P.f: mixed co-infection of Ascaris, Trichuris and Plasmodium, Hb^a: arithmetic mean haemoglobin levels in g/dl in non infected individuals, Hb^b: arithmetic mean haemoglobin levels in g/dl in infected individuals, A: Anaemia cases, MPD: geometric mean Plasmodium parasite densities in parasites per μl of blood, MAD: geometric mean Ascaris parasite densities in eggs per gram of faeces, MTD: geometric mean Trichuris parasite densities in eggs per gram of faeces. P-values <0.05 are significant statistically; p^a, p^b, and p^c are p-values among villages, sex and age groups, respectively.
most the anaemic cases were classified as moderate anaemia with a mean haemoglobin level of 9.8 ± 0.8 g/dl. Only one case of severe anaemia (0.9%) was observed with a total haemoglobin level of 6.7 g/dl. Fifty three (40.5%) and 57 (43.5%) participants were found to be anaemic in Nkassomo and Vian, respectively. Although anaemia did not differ by locality and sex, the prevalence of anaemia decreased significantly with the age groups (p = 0.003) (Table 1).

Overall, all anaemic cases were found to carry at least one of the detected parasitic infections. There were 13 (52%), 95 (46.8%), and 19 (38%) cases of anaemia amongst those infected with T. trichiura, P. falciparum, and A. lumbricoides, respectively. The prevalence however was observed to increase by up to 71% in co-infection cases (Figure 2). Although anaemia was more frequent in those infected with T. trichiura and P. falciparum as co-infections, A. lumbricoides did not show any influence on the prevalence of anaemia in co-infections. Exploratory multiple linear regression analysis depicted positive associations between the parasite densities (P. falciparum and T. trichiura) considered as the independent variables with anaemia and fever, considered as the dependent variables. However, these infections were not significant predictors of anaemia and fever in the area.

Discussion

Results of this study provided preliminary data on the epidemiology of malaria and geohelminthiasis in Nkassomo and Vian, two rural communities of the Mfou health district in the Centre region of Cameroon. Malaria in these communities is high and mainly due to Plasmodium falciparum. The overall observed prevalence of 77.2% is higher than the 40.6% recorded in 2009 amongst schoolchildren in the same health district [12]. This difference is not unlikely since our study population was larger. This observed prevalence is equally higher than those obtained in other regions of the country and elsewhere [7,9,22,32] and could be linked to differences in the geological and climatic conditions that might influence malaria vector breeding and distribution in different areas. Although the mean intensity of P. falciparum malaria can reach the severe level (>5,000 p/μl) in most regions where malaria is endemic [10], our study area revealed a very low parasite densities (<500 p/μl). Malaria intensity was therefore qualified as light according to Trape et al. [33] classification, and this could be attributed to the difference between the period of infection and the survey, the infection rate, and the preventive measures (chemophrophylaxis or auto-medication at individual level) considered as the main factors that can influence the infection intensity [34]. Although malaria is usually shown with highest prevalence among children and pregnant women, our study revealed that pre and young schoolchildren (0–9 yrs) had the highest prevalence and intensities of malaria, which did not differ significantly amongst age groups. This is probably due to hyper transmission of the infection in this locality and the acquisition of plasmodial immunity with increasing age after 9 years [2,11,32]. In fact, as stated previously, parasite distribution is generally not homogenous and malaria can affect the age with

| Type of infection | P. falciparum (n = 203) | Yes | 37.3 ± 0.6 | 66 (32.5) | 137 (67.5) | 0.000 |
|-------------------|------------------------|-----|------------|-----------|-----------|------|
| A. lumbricoides (n = 50) | No | 12 | 37.4 ± 0.4 | 5 (21.7) | 7 (32.5) | 0.047 |
| T. trichiura (n = 25) | Yes | 20 | 37.1 ± 0.5 | 9 (43.6) | 11 (53) | - |
| A. lumbricoides-T. trichiura (n = 9) | Yes | 7 | 37.4 ± 0.2 | 5 (21.7) | 2 (28.6) | - |
| No | 2 | 37.6 ± 0.1 | 2 (100) | - |

MAT = arithmetic mean of axial temperature ± Standard Deviation in degree Celsius. Proportions are expressed as number of cases with percentages in brackets. P-values between fever and asymptomatic malaria cases are given.

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Table 2. Distribution of Plasmodium falciparum and malaria symptoms amongst individuals with geohelminth infections.

| Parasite species | Mean Parasite density | p-value |
|------------------|-----------------------|---------|
| Single infections | Single co-infection | Mixed Co-infection |
| A. l-P. f (n = 38) | T. t-P. f (n = 20) | A. l-T. t-P. f (n = 7) | p* | p^b |
| A. lumbricoides (n = 50) | 179 (24, 1920) | 257 (24, 3552) | - | 501 (96, 3552) | 0.047 | 0.000 |
| T. trichiura (n = 25) | 35 (24, 72) | - | 42 (24, 96) | 29 (24, 48) | 0.797 | 0.374 |
| P. falciparum (n = 203) | 107 (40, 4000) | 143 (40, 2360) | - | 99 (40, 1280) | 0.018 | 0.776 |

Parasite densities are given as geometric mean values with minimum and maximum values in brackets, and expressed in eggs per gram of faeces (eps) for Ascaris and Trichuris, or in parasites per micro litre of blood (p/μl) for Plasmodium. P-values <0.05 are statistically significant; p* p-values between single infections and single co-infections, p^b p-values between single infections and mixed co-infections.

Table 3. Comparison of geometric mean parasite densities between single infections and co-infections.

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Helminth parasites are highly prevalent in rural communities due to poor sanitary conditions prevailing in most of these areas [9]. In order to assess the prevalence of these infections in Mfou rural areas, our study recorded an overall prevalence of 29.6% for geohelminth infections, with *A. lumbricoides* as the main parasite species, followed by *T. trichiura*. Although similar findings were obtained for earlier studies on schoolchildren in the same region in 2009 [12], it did not corroborate with those in the South-West region of the country and Ethiopia [1,15]. This observation is not uncommon as helminths' distribution is generally known to vary with environmental conditions that are directly related to the level of healthiness of each area [13,36]. No case of any other geohelminths (Hookworms *spp.*, *S. stercoralis*) was found. However, as hookworm ova is likely deform and may clear within 24 hours following Kato Katz smear preparation [31], the absence of Hookworms *spp.* could stand as a possible bias for this study due to the time spent (7–9 hours) between the preparation and examining of the Kato Katz smears. Knowing that *A. lumbricoides* infection is usually associated with higher parasite intensity in contrast to *T. trichiura* infection, and according to the WHO classification of helminth parasite densities [37], this study revealed that both parasites are characterized by light intensity in Mfou. This low intensity and prevalence of intestinal parasites in Mfou rural areas could be attributed to the multiple deworming campaigns that the government implemented in the region the year before this study [17]. In addition, can be the chemoprophylaxis applied at individual level as preventive measure. This auto medication seemed to be important in Vian where the intensities of *A. lumbricoides* were significantly higher compared to Nkassomo. The proximity of Nkassomo to the lone health facility in the health area could as well be central to this observed difference as there is decreased access of the population of Vian to appropriate drugs in case of infection. Similar results have been observed in two connected villages in previous studies by Nkengazong et al. [15]. However, the infection rate of these species did not differ by locality. Although males were more vulnerable to *T. trichiura* infection, *A. lumbricoides* infection was significantly different amongst age groups with school children (5–14 yrs) having the highest prevalence of *Ascarisis* (p = 0.002) and total geohelminth infection (p = 0.008). This shows that geophagia or soil ingestion is more important in males and school children who are usually in contact with the soil, the main source of contamination for soil-transmitted helminths or geohelminths. Similar results were observed by Hamit et al. [38] and Tchinda et al. [12]. On the other hand, the World Health Organization reported that 70% of total geohelminth morbidity could be avoided when treating only school children (WHO, 2004). To achieve this, sanitary education of the population is crucial. Any control or deworming strategy of geohelminths should therefore be preceded by a full sensitization and awareness of the population about individual hygiene, which is key to any preventive measures against these infections.

So far, very little is known about the distribution and impact of intestinal worm co-infections across Cameroon. This study revealed that many malarial patients were infected by at least a geohelminth parasite overall prevalence of 22.1%. Observably, *P. falciparum* and *A. lumbricoides* were the main parasite species for malaria and geohelminthiasis and constituted the main co-infection. This was followed by *T. trichiura-P. falciparum* co-infection and mixed co-infection. Our data showed that co-infection had the same distribution with single infections between localities, and that increasing endemicity of single infections is likely to enhance of the establishment of co-infections. In fact, schoolchildren aged 5–14 years had significantly higher prevalence...
of co-infection (p = 0.001), due of increased susceptibility of this group to A. lumbricoides as reported elsewhere [1,12,16,22,39–41]. In contrast to single infections, association between T. trichiura and P. falciparum was significantly higher in males due to increased susceptibility of men to trichinosis [30]. Thus, treatment of cases of co-infections should rely on the association of measures used to fight against single infections [22].

Co-infected parasites do not necessarily act independently as a consequence of their co-existence in the same host. Indeed, the increase intensities of P. falciparum and A. lumbricoides in their co-infection was significant. However, akin to earlier studies, the observed influence of A. lumbricoides on P. falciparum infection in this study was important though not statistically significant due to a decrease in anti-malarial immunity by concomitant helminth infections [1,19,20,21,42]. It has also been shown that the risk of developing severe malaria increases in children co-infected with Ascariasis due to an increase in malaria intensity [43]. However, the mechanisms of such interactions remain unknown. In contrast to A. lumbricoides, T. trichiura parasite was associated with a decrease in intensity of P. falciparum at a non significant level. Nevertheless, interactions between T. trichiura and P. falciparum seemed be higher than those between A. lumbricoides and P. falciparum in the mixed co-infection when compared to single co-infections (Table 3). A direct consequence of such interactions was observed in the distribution of anaemia amongst the study population.

Overall, all cases of anaemia were carriers of at least one infection with co-infection being the main parasitic factor of anaemia, followed by single infections of T. trichiura, P. falciparum, and A. lumbricoides. The intensity of anaemia was respectively lower and higher than that obtained by Ehrhardt et al. [35] and Degarege et al. [1], who attributed this to the above parasites including Hookworms spp. However, it is important to note that these are interesting factors of anaemia in the study area but not probably the only. Further studies should consider other factors as other infections (e.g., Schistosomiasis, Ankylostomiasis), nutritional status, socio-economic level, etc. Anaemia did not significantly differ by locality and sex, but total haemoglobin level increased with age, with children less than five years having the highest prevalence of anaemia probably due to their high infection rate for P. falciparum and T. trichiura parasites. However, age was not a predictor of anaemia in our study area in contrast to Nkou-Akenji et al. in South-West Cameroon [10]. According to above observations, this decrease in prevalence of anaemia by age could be linked to acquisition of adequate immunity against responsible infections [23]. The prevalence of anaemia was observed to increase in co-infections compared to single infections, probably due to interactions between T. trichiura and P. falciparum; because A. lumbricoides does not seem to have any impact on haemoglobin level in co-infections as reported by Kouontcho et al. [4]. This increase in prevalence of anaemia in co-infections is due to additive effects in the mechanisms of these infections on total haemoglobin concentration. In fact, T. trichiura is associated with chronic loss of blood and iron in gastrointestinal tube, while P. falciparum is associated with bursting and loss of red blood cells during its blood stage, all contributing to haemoglobin loss and anaemia. However, unlike in the southwest region of the country, the intensities of these parasites were not predictors of anaemia in Nkassomo and Vian, [10].

Conclusion
The findings suggest that malaria is hyperendemic in the study localities, and co-exists with geohelmints with their co-infections common amongst schoolchildren. This co-infection constitutes an important risk to anaemia while exacerbating malaria intensity. Though further epidemiological studies are needed to support these observations and assess the mechanisms involved in such interactions, the results provide useful information necessary to design control management strategies for malaria in the context of co-infection.

Supporting Information

Checklist S1 STROBE checklist. (DOC)

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Author Contributions
Conceived and designed the experiments: VHMT, JDB RSM. Performed the experiments: VHMT FZ CHTS GA VM AM. Analyzed the data: VHMT FZ CHTS. Contributed reagents/materials/analysis tools: VHMT JDB RSM. Wrote the paper: FZ CHTS JDB VHMT.

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