Malaria and intestinal parasite co-infections and impact on haematological parameters among people living with HIV in Buea, Southwest Cameroon

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Abstract

Background: Both malaria and intestinal parasites are endemic in Cameroon, and their co-infection can be of great impact on haematological parameters among people living with HIV (PLWH). This cross-sectional study determined the prevalence and impact of malaria/intestinal parasite co-infections on haematological parameters in PLWH and HIV negative individuals in Buea, Cameroon from March-August 2019.

Methods: The study population (500) comprised of 190 PLWH, 216 HIV consenting negative individuals from the Buea community and 94 HIV negative patients at the Buea Hospital out-patient department (OPD). Participants were examined clinically, and the collected blood sample was used for malaria parasite (MP) detection, HIV diagnosis and full blood count analysis. Stool samples were examined for the detection of intestinal parasites (IPs) using the formol-ether concentration and modified Zheil-Neelsen techniques. Proportions were compared using Pearson's Chi-square test and association of anaemia with the independent variables was evaluated using multivariate logistic regression analysis

Results: Overall, MP, IPs and MP/IPs co-infection prevalences were 17.0% (85), 13.0% (65) and 2.2% (11) respectively. When compared with HIV negative participants from the communities, PLWH had the highest prevalence of MP (16.3%, $P = 0.17$), IPs (23.7%, $P \leq 0.001$) and MP/IPs co-infection (3.7%, $P = 0.04$). *Plasmodium falciparum* was the only malaria parasite identified. Detected protozoa (10.2%) were *Cryptosporidium* species and *Entamoeba histolytica* while detected helminths (3.8%) were *Ascaris lumbricoides*, *Schistosoma mansoni*, hookworm, *Dipylidium caninum*, *Hymenolepis nana* and *Taenia* species. *Cryptosporidium* species (16.8%, 32) was the most prevalent protozoan IP while hookworm (2.6%, 5) was the most prevalent helminth among PLWH. The significant risk factors associated with anaemia included being aged 21-30 years ($P=0.029$), a male ($P<0.001$), MP positive ($P<0.001$), HIV positive ($P<0.001$) and feverish ($P<0.001$). Participants co-infected with HIV, MP and IP had a significantly lower mean haemoglobin value (10.56 ± 1.21 g/dl).

Conclusion: Malaria and intestinal parasites are still public health concern among PLWH and co-infections have negative influence on haematological parameters especially haemoglobin concentration. Routine screening for malaria and IPs in PLWH is recommended, as well as haemoglobin concentration monitoring.

Background

Parasitic infections are a major cause of morbidity and mortality in Africa; especially in resource limited tropical and sub-tropical regions like sub-Saharan Africa [1] where the majority of HIV/AIDS cases are concentrated. People living with HIV (PLWH) are threatened by a great number of diseases caused by different types of biological agents.

The number of new HIV infections globally continued to decline in 2017, from 3.4 million cases in 1996 to 1.8 million cases in 2017 [2]. HIV infection is characterized by immunosuppression that exposes the

infected individual to a variety of microbial and parasitic infections such as *Plasmodium*, *Mycobacterium tuberculosis* and intestinal parasitic infections. These infections (opportunistic or not) are a serious threat to the health status of people living with HIV, especially in developing countries like Cameroon. To achieve HIV epidemic control in 2021, the Cameroon government has been implementing various strategies that led to an encouraging increase in anti-retroviral therapy (ART) coverage (53%) and viral load (VL) suppression (44.7%) nationally in 2019 [3].

WHO African region still bears the largest burden of malaria morbidity (93%), with *Plasmodium falciparum* accounting for 99.7% of malaria cases in 2018 [4]. WHO reported Cameroon as the fourth Sub-Saharan African country behind Nigeria, Democratic Republic of Congo and Mozambique responsible for the malaria cases in the region [5]. Many studies have consistently reported an association between HIV infection and malaria in Cameroon [6, 7, 8, 9, 10]. In Cameroon, each region shows varied prevalence of both malaria and HIV infection. HIV prevalence is approximately 3.7% [3] while malaria transmission is stable throughout the year, with an overall malaria test positivity rate of 62% registered in 2017 [11].

The burden of intestinal parasite infections (IPIs) is greatest in low-income areas and are major public health problems in certain areas of Sub-Saharan Africa. These IPIs are associated with humid climate, unsanitary environments and poor socio-economic conditions. Parasites causing these IPIs are protozoa (*Cryptosporidium species*, *Cyclospora cayetanensis*, *Isospora belli*, and *Microsporidia spp*, *Entamoeba histolytica*) and helminthes (*Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, *Schistosoma mansoni*, *Taenia species*). *Cryptosporidium species*, *Cyclospora cayetanensis*, *Isospora belli*, and *Microsporidia spp* are often referred to as opportunistic parasites in people living with HIV and cause great morbidity when CD4 T cell count falls below 200 cells/ μ l [12]. Various studies carried out among African adults living with HIV have reported IPI rates ranging from 10–45% [13, 14]. Majority of the research on the impact of IPIs on HIV progression in Cameroon, occurred before the wide availability of ART [15] hence, the need for further evaluation.

In Cameroon, PLWH face similar environmental realities as those not infected with HIV and so can easily get co-infected with combinations of intestinal and malaria parasites. Such co-infections may have considerable health consequences which could lead to severe clinical symptoms and pathology in PLWH. Studies have shown that individuals co-infected with more than one parasite species have an increased risk of developing more frequent and severe diseases due to interactions among infecting parasite species [16, 17, 18].

Malaria and intestinal parasites in PLWH can both lead to severe haematological abnormalities. Although, ART in combination with cotrimoxazole confers a level of immunity to infections in PLWH, it has been reported to have adverse effects on the red blood cell indices of these patients, thereby causing anaemia [19]. Although malaria and intestinal parasites as well as ART are known etiological factors in anaemia [20, 21, 15, 10], the extent to which their interaction might enhance the risk of the haematological abnormalities merits further investigations. Previous studies have reported on the

influence of malaria/HIV co-infection on the occurrence of anaemia [22, 18, 10]. The situation regarding PLWH co-infected with malaria and intestinal parasites in the area following the intensification of different control strategies such free availability of ART and cotrimoxazole, access to viral load testing and community ART dispensation is unknown. Therefore, the present study was undertaken to determine the prevalence of malaria parasite and intestinal parasites in PLWH compared to HIV negative counterparts as well as to assess the effects of these co-infections on clinical and haematological parameters in order to provide relevant information to policy makers in the country.

Methods

Study area and participants

Participants were enrolled from the Buea Regional Hospital (HIV Treatment centre and Out-patient unit) and from different neighbourhoods in Buea. Buea has a population of about 200,000 inhabitants and it is the South West Regional capital. The Buea Regional hospital is the referral HIV treatment centre in Fako Division serving the population. With the presence of the Mount Cameroon, Buea is a major touristic site and has a business-friendly environment. Its inhabitants are from almost all ethnic groups of Cameroon. In the national HIV prevalence ranking, the South West Region is the 6th Region with a prevalence of 3.6% which equates the national prevalence (3.7%) [23] as well as the second to the last Region (33.8%) with the least viral load suppression. Buea is an area of moderate malaria transmission, with peak time for malaria transmission during and just after the rainy season (March to September) [24]. Its tropical climate provides a suitable environment for the development of the malaria vectors and this therefore contributes to increased malaria transmission. There is also inadequate access to safe water in this region, predisposing the population to water-borne infections including IPIs.

Three categories of participants; PLWH at the HIV Treatment centre, HIV-negative individuals on consultation at the outpatient department (OPD) and HIV-negative individuals in different neighbourhood were enrolled in the study. Participants were recruited irrespective of their age, sex, marital status, occupation, level of education; with or without malaria-related and intestinal parasite infections-related signs and symptoms, ART regimen and duration on ART. Only participants who signed the informed consent form and willingly accepted to be counselled and tested for malaria, IPIs and HIV were enrolled in the study. Participants on antibiotics, malaria treatment or antiparasitic agents two weeks prior to the sample collection were excluded from the study.

Study Design And Sample Size

This study was a hospital and community-based cross-sectional study carried out within April 2018 – February 2019. HIV-negative participants included persons who consented to be screened for HIV during the process and were negative while apparently healthy HIV-negative individuals were those enrolled through HIV outreach programs in their homes and offices in the Buea neighbourhoods (Molyko,

Bonduma, Great Soppo, GRA, Buea town, Bokwaongo, Bokova, Mile 18, Clerks and Federal quarters). The sample size was determined using the formula $n = Z^2pq/d^2$ [25] where n represented the sample size evaluated, Z was 1.96, which is the standard normal deviate (for the 95% confidence interval, CI), p was 14.1% [10] and 82.6% [15], the prevalence of malaria and IPIs in PLWH and HIV negative participants respectively, q was 1-p and d was 0.05. The sample size calculated for malaria parasite prevalence was 186 while that for IPIs prevalence was 220 given an optimum sample size for the study of 406 participants.

Data Collection

Questionnaire

A pre-structured questionnaire was used to collect socio-demographic information such as age, sex, location of residence, level of education, marital status, occupation while clinical data such as ART usage, duration on treatment, ART regimen, viral load (VL) were collected from each HIV patient's file at the HIV centre. Health and preventive practices towards malaria and IPIs as well as behavioural habits regarding health conditions with a history of symptoms were recorded. Environmental conditions such as the water supply, swimming practices and animal ownership were also recorded for all the patients.

Sample Collection And Processing

Participants were instructed on how to collect stool sample in universal transparent faecal containers with a screw cap lid. The samples were transported to the Malaria Research Laboratory of the University of Buea. On arrival in the laboratory, the stool samples were analysed macroscopically and preserved in 10% formalin for 24 hours for further analysis. Formol-ether concentration technique and modified Ziehl-Neelsen (ZN) method [26] were performed for the detection of intestinal parasites (protozoa and helminths).

After provision of a stool sample, the participant's body temperature was recorded using a digital thermometer and fever was defined as temperature $\geq 37.5^\circ\text{C}$. Blood was collected under sterile conditions into two well-labelled Ethylenediaminetetraacetate (EDTA) tubes for each participant for HIV screening and confirmation, malaria diagnosis and full blood count (FBC). Part of the blood was used to prepare thick and thin blood films on a labelled slide that was Giemsa-stained and analysed following standard procedures [26] by two experienced microscopists. The blood in one of the tubes was used for HIV rapid test (in the case of presumed HIV negative participants), CD4 T-cell count and to conduct FBC. Viral loads were obtained from the HIV patient's file at the HIV Care Centre. Viral load ≤ 50 copies /mL of blood was considered undetectable [27].

Haematological Analysis

The FBC analysis was carried out using the URIT-3300 automated haematology analyser (URIT Medical Electronic CO., LTD. Jiu Hua Road, Gungxi, China), following the manufacturer's instructions. Values of the following parameters were obtained from the haematology analyser: White blood cell (WBC), red blood cell (RBC) and platelet counts, haemoglobin concentration (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC). Anaemia was defined as haemoglobin concentrations below the World Health Organization (WHO) reference values corresponding to age or gender [28]. Leucopenia was defined as WBC < 4000 cells/ μ L, microcytosis as MCV less than 80 fL [29] and thrombocytopenia was defined as platelet count < 150 000 cells/ μ L. CD4T cell counts were determined using the BD FACS Count™ System (BD Biosciences, USA) as per the manufacturer's instructions. To assure the quality of the laboratory data, standard operating procedure for each test was followed. Reagents were checked for expiry date and prepared according to the manufacturer's instruction. The Determine Rapid Test Kit (Abott Laboratories, CO., Ltd. Minato-Ku, Tokyo Japan) was used to test the supposed HIV negative subjects for HIV-1 following the manufacturer's instruction.

Statistical Analysis

Data was analysed using IBM-Statistical Package for the Social Science (SPSS) version 20.0 software (SPSS Inc., Illinois, USA). Baseline characteristics was summarized as mean with 95% CI and compared using the independent *t*-test. Malaria and intestinal parasites data were summarized into means and standard deviation (SD) and percentages were used in the evaluation of the descriptive statistics. The significance of differences in prevalence was explored using Pearson's Chi-square while Analysis of Variance (ANOVA) or Student's *t* test were used where appropriate to assess difference in group means. The association of anaemia with the independent variables was evaluated using multivariate logistic regression analysis. $P < 0.05$ was considered for statistical significance.

Ethical Considerations

Using an information sheet, a brief talk was given to the participants in English language (exceptionally in Pidgin English) and the participants were then invited to participate in the study by signing an informed consent form. In the case of children (≤ 15 years), their parents signed proxy informed consent form on their behalf. Ethical clearance was obtained from the Institutional Review Board hosted by the Faculty of Health Sciences of the University of Buea (Reference Number: 661-09). For confidentiality purposes, patients were identified by special identification numbers. Results of participants with parasitic infections in the neighbourhoods were sent as soon as possible to a clinician for their case management.

Results

Socio-demographic and clinical characteristics of the participants

Six hundred and eighty three (683) participants were approached to participate in the study, with 500 (73.2%) being successfully enrolled. Out of the 500 participants, 190 were HIV positive patients from the HIV Treatment Centre, 216 were HIV negative from the communities and 94 were HIV negative from the OPD of the Buea Regional Hospital as shown in Fig. 1.

Overall, the mean (SD) age of the participants was 30.0 (17.1) years. There were more females (66.0%) than males (34.0%). A greater proportion of the study population belonged to the ≤ 20 years age group (31.6%) and the least to the 31–40 years age group (17.6%) as shown in Table 1. Majority of the PLWH had primary level of education (66.3%), were self-employed (31.2%) and were single (62.0%). Among HIV negative participants (in communities and at OPD respectively), majority were students (56.5%, 56.5%) and were single (63.0%, 51.0%) as revealed in Table 1.

Table 1
Socio-demographic profile of the study population

Parameters	HIV Positive	Communities HIV Negative	OPD HIV Negative	Overall
	n (%)	n (%)	n (%)	n (%)
Age groups (years)				
≤ 20	42 (22.1)	72 (33.3)	44 (46.8)	158 (31.6)
21–30	20 (10.5)	70 (32.4)	22 (23.4)	112 (22.4)
31–40	48 (25.3)	28 (13.0)	12 (12.8)	88 (17.6)
> 40	80 (42.1)	46 (21.3)	16 (17.0)	142 (28.4)
Mean ± SD age	35.5 ± 16.5	27.6 ± 15.8	24.0 ± 17.9	30.0 ± 17.1
Sex				
Female	140 (73.7)	138 (63.9)	52 (55.3)	330 (66.0)
Male	50 (26.3)	78 (36.1)	42 (44.7)	170 (34.0)
Educational Level				
No formal	7 (3.7)	18 (8.6)	22 (23.9)	47 (9.5)
Primary	126 (66.3)	54 (25.7)	28 (30.4)	208 (42.3)
Secondary	43 (22.6)	64 (30.5)	18 (19.6)	125 (25.4)
Tertiary	14 (7.4)	74 (35.2)	24 (26.1)	112 (22.8)
Occupation				
Student	48 (25.4)	122 (56.5)	52 (56.5)	222 (44.7)
Unemployed	25 (13.2)	12 (5.2)	12 (13.0)	49 (9.9)
Self-employed	59 (31.2)	28 (13.0)	8 (8.7)	95 (19.1)
Employee	57 (30.2)	54 (25.0)	20 (21.7)	131 (26.4)
Marital status				
Single	93 (62.0)	102 (63.0)	25(51.0)	220(60.9)
Married	61 (38.0)	60 (37.0)	24(49.0)	141(39.1)
ITN usage	109 (57.4)	76 (35.2)	38 (40.4)	223 (44.6)

Axillary temperature measurement revealed 15.0% of the general population were feverish, with the highest proportion (19.1%) among HIV negative patients at the OPD. Clinical signs (headache, joint pains,

nausea, vomiting etc...) were less frequent among HIV negative participants from the communities than among HIV negatives at the OPD (40.5%). Anaemia occurred in 56.6% (283) of the participants and PLWH had the highest occurrence (76.8%) when compared with their HIV negative counterparts. Among PLWH, half of the population (50.0%) had CD4 T cell count between 200–500 cells/ μ L of blood, majority (97.9%) were on ART, 91.9% (171) were taking first line ART regimen and 77.5% (141) had attained viral suppression($\leq$ 50 copies/mL of blood) as shown in Table 2.

Table 2
Clinical characteristics and laboratory profile of the study population

Parameters	HIV Positive n (%)	Communities HIV Negative n (%)	OPD HIV Negative n (%)	Overall n (%)
Fever status Febrile	23 (12.1)	34 (15.7)	18 (19.1)	75 (15.0)
Afebrile	167 (87.9)	182 (84.3)	76 (80.9)	425 (85.0)
Mean temperature (SD) in °C	37.1 (0.5)	37.1 (0.5)	37.3 (0.5)	37.1 (0.5)
Presence of clinical signs Yes	39 (23.5)	32 (18.2)	30 (40.5)	101 (24.3)
No	127 (76.5)	144 (81.8)	44 (59.5)	315 (75.7)
Anaemic status Anaemic	146 (76.8)	86 (39.8)	51 (54.3)	283 (56.6)
Non-anaemic	44 (23.2)	130 (60.2)	43 (45.7)	217 (43.4)
Mean Hb (SD) in g/dL	11.1(1.5)	12.5 (2.1)	12.1 (2.8)	11.9 (2.2)
CD4 T-cell count(cells/ μ L) < 200	13 (12.5)	-	-	13 (12.5)
200–500	52 (50.0)	-	-	52 (50.0)
> 500	39 (37.5)	-	-	39 (37.5)
Mean CD4 T cell count SD (cells/ μ L)	466.0 (275.9)	-	-	466.0 (275.9)
ART usage Yes	186 (97.9)	-	-	186 (97.9)
No	4 (2.1)	-	-	4 (2.1)
ART regimen First line	171 (91.9)	-	-	171 (91.9)
Second line	15 (8.1)	-	-	15 (8.1)
ART Duration (years) < 1	36 (19.4)	-	-	36 (19.4)
1–3	61 (32.8)	-	-	61 (32.8)
> 3	89 (47.8)	-	-	89 (47.8)

Parameters	HIV Positive n (%)	Communities HIV Negative n (%)	OPD HIV Negative n (%)	Overall n (%)
Viral load (RNA copies/ml)		-	-	
Not detected (≤ 50)	141 (77.5)	-	-	141 (77.5)
≥ 50	41 (22.5)	-	-	41 (22.5)

Malaria Parasite Prevalence

Out of the 500 participants, 85 (17.0%) were positive for MP with *Plasmodium falciparum* as the only species observed. Although not statistically significant ($\chi^2 = 3.52$ $P = 0.17$), MP prevalence was higher in HIV negatives at the OPD (23.4%) and least in HIV negatives in the communities (14.8%) as shown in Fig. 2.

With respect to gender and age MP prevalence was significantly higher in HIV negative females in the OPD (30.8%) and the HIV negatives > 40 years in the communities (26.1%) than their respective counterparts. Overall, the prevalence of MP was significantly higher in those febrile (85.3%) and those anaemic (23.0%) than their respective counterparts. Specifically, significantly higher prevalence of MP was observed in those febrile among PLWH (87.0%), HIV negatives in the communities (82.4%) and HIV negatives in the OPD (88.9%). Similar trend in significantly higher prevalence of MP was observed in those anaemic among HIV negative participants in the communities and at the OPD ($P \leq 0.001$, $P \leq 0.001$) as shown in Table 3. With respect to the presence of clinical signs, malaria parasite was most prevalent in HIV negative participants presenting themselves at the OPD (33.3%) unlike their contemporaries ($\chi^2 = 4.09$, $P = 0.04$). Those who attained viral suppression and those with CD4 T-cell count between 200–500 cells/ μ l had the highest prevalence of MP (17.0% and 21.6% respectively) although the difference was not statistically significant (Table 3).

Table 3

Malaria parasite prevalence among the different categories of patients with respect to socio-demographic and clinical data

Categories	HIV Positive		Community HIV Negative		OPD HIV Negative		Overall	
	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)
Gender Male	50	18.0 (9)	78	15.4(12)	42	9.5 (4)	170	14.7 (25)
Female	140	15.7 (22)	138	14.5 (20)	52	30.8 (16)	330	17.6 (58)
Level of Significance	$\chi^2 = 0.18, P = 0.41$		$\chi^2 = 0.31, P = 0.50$		$\chi^2 = 6.26, P = 0.01^*$		$\chi^2 = 0.62, P = 0.25$	
Age ≤ 20	42	16.7 (7)	72	13.9 (10)	44	18.2 (8)	158	15.8 (25)
21–30	20	20.0 (4)	70	5.7 (4)	22	36.4 (8)	112	14.3 (16)
31–40	48	12.5 (6)	28	21.4 (6)	12	16.7 (2)	88	15.9 (14)
≥ 40	80	17.5 (14)	46	26.1 (12)	16	12.5 (2)	142	19.7 (28)
Level of Significance	$\chi^2 = 0.86, P = 0.84$		$\chi^2 = 10.83, P = 0.01^*$		$\chi^2 = 3.54, P = 0.32$		$\chi^2 = 1.40, P = 0.70$	
Fever status Febrile	23	87.0 (20)	34	82.4 (28)	18	88.9 (16)	75	85.3 (64)
Afebrile	167	6.6 (11)	182	2.2 (4)	76	5.3 (4)	425	4.5 (19)
Level of Significance	$\chi^2 = 95.03, P \leq 0.001^*$		$\chi^2 = 145.84, P \leq 0.001^*$		$\chi^2 = 60.76, P \leq 0.001^*$		$\chi^2 = 300.42, P \leq 0.001^*$	
Clinical signs Yes	39	20.5 (8)	32	6.2 (2)	30	33.3 (10)	101	19.8 (20)
No	127	15.0 (19)	144	13.9 (20)	44	13.6 (6)	315	14.3 (45)

* Statistically significant P value

Categories	HIV Positive		Community HIV Negative		OPD HIV Negative		Overall	
	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)
Level of Significance	$\chi^2 = 0.67, P = 0.27$		$\chi^2 = 1.39, P = 0.19$		$\chi^2 = 4.09, P = 0.04^*$		$\chi^2 = 1.76, P = 0.12$	
Anaemic status Anaemic	146	17.1 (25)	86	25.6 (22)	51	35.3 (18)	283	23.0 (65)
Non-anaemic	44	13.6 (6)	130	7.7 (10)	43	4.7 (2)	217	8.3 (18)
Level of significance	$\chi^2 = 0.22, P = 0.41$		$\chi^2 = 10.57, P = 0.001^*$		$\chi^2 = 12.36, P = 0.001^*$		$\chi^2 = 16.74, P = 0.001^*$	
Viral load (copies/ml) Not detected (≤ 50)	141	17.0 (24)					141	17.0 (24)
≥ 50	41	14.6 (6)					41	14.6 (6)
Level of Significance	$\chi^2 = 0.13, P = 0.46$						$\chi^2 = 0.13, P = 0.46$	
CD4 T-cell count (cells/ μ l) ≤ 200	15	6.7 (1)					15	6.7 (1)
200–500	51	21.6 (11)					51	21.6 (11)
≥ 500	40	15.0 (6)					40	15.0 (6)
Level of significance	$\chi^2 = 2.00, P = 0.36$						$\chi^2 = 2.00, P = 0.36$	
ART usage Yes	186	16.7 (31)					186	16.7 (31)
No	4	0.0 (0)					4	0.0 (0)

* Statistically significant P value

Categories	HIV Positive		Community HIV Negative		OPD HIV Negative		Overall	
	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)	N	Malaria parasite positive % (n)
Level of significance	$\chi^2 = 0.79, P = 0.48$						$\chi^2 = 0.79, P = 0.48$	
ART duration ⊠1	36	13.9 (5)					36	13.9 (5)
1–3	61	18.0 (11)					61	18.0 (11)
> 3	89	16.9 (15)					89	16.9 (15)
Level of significance	$\chi^2 = 0.28, P = 0.86$						$\chi^2 = 0.28, P = 0.86$	
ART regimen First line	171	17.5 (30)					171	17.5 (30)
Second line	15	6.7 (1)					15	6.7 (1)
Level of significance	$\chi^2 = 1.21, P = 0.24$						$\chi^2 = 1.21, P = 0.24$	
* Statistically significant P value								

Intestinal Parasite Prevalence

Among the 500 participants, 65 (13.0%) were positive for IPIs with a higher occurrence of protozoa (10.4%) than helminths (3.8%). The prevalence of IPIs was highest among PLWH (23.7%) and least among HIV negative controls in the communities (5.6%) and the difference was significant at $\chi^2 = 31.73$, $P \leq 0.001$ as shown in Fig. 3.

Detected protozoa were *Cryptosporidium species* and *Entamoeba histolytica* while detected helminths were *Ascaris lumbricoides*, *Schistosoma mansoni*, hookworm, *Dipylidium caninum*, *Hymenolepis nana* and *Taenia* species. When comparing infection prevalence among the different groups, PLWH were the most infected both with helminths (6.8%) and protozoa (19.5%) and the differences were statistically significant ($\chi^2 = 9.74$, $P = 0.008$; $\chi^2 = 28.78$, $P \leq 0.001$ respectively) as shown in Table 4.

Table 4
Prevalence of intestinal parasites in the study population

Parasites Species	HIV Positive n (%)	Community HIV Negative n (%)	OPD HIV Negative n (%)	Overall population n (%)	Chi Square P Value
Protozoa					
At least one protozoa	37 (19.5)	10 (4.6)	4 (4.3)	51(10.2)	$\chi^2 = 28.78$ P = 0.001*
<i>Cryptosporidium spp</i>	32 (16.8)	10 (4.6)	4 (4.3)	46 (9.2)	$\chi^2 = 21.63$ P = 0.001*
<i>Entamoeba histolytica</i>	6 (3.2)	0 (0.0)	0 (0.0)	6 (1.2)	$\chi^2 = 9.96$ P = 0.01*
Helminths					
At least one helminth	13 (6.8)	2 (0.9)	4 (4.3)	19 (3.8)	$\chi^2 = 9.74$ P = 0.008*
<i>Ascaris lumbricoides</i>	4 (2.1)	0 (0.0)	2 (2.1)	6 (1.2)	$\chi^2 = 4.63$ P = 0.09
Hookworm	5 (2.6)	0 (0.0)	0 (0.0)	5 (1.0)	$\chi^2 = 8.28$ P = 0.02*
<i>Schistosoma mansoni</i>	1(0.5)	0 (0.0)	2 (2.1)	3 (0.6)	$\chi^2 = 4.98$ P = 0.08
<i>Strongyloides stercoralis</i>	1(0.5)	0 (0.0)	0 (0.0)	1 (0.2)	$\chi^2 = 1.64$ P = 0.44
Tapeworm	2 (1.1)	2 (0.9)	0 (0.0)	4 (0.8)	$\chi^2 = 0.96$ P = 0.62
* Statistically significant P value					

Infection with *Cryptosporidium* species was the most common IPs identified in the study population (9.2%, 46), followed by *Ascaris lumbricoides* (1.2%, 6) and *Entamoeba histolytica* (1.2%, 6) and then hookworm (1.0%, 5). When compared with their counterparts, PLWH had the highest prevalence of

Cryptosporidium species (16.8%), *Entamoeba histolytica* (3.2%) and hookworm (2.6%) and the differences were significant ($P \leq 0.001$, $P = 0.01$, $P = 0.02$ respectively) as shown in Table 4.

Malaria /intestinal Parasite Co-infections

Malaria/IPs co-infection prevalence was 2.2% (11) and was significantly higher among PLWH (3.7%) when compared with HIV negative participants in the communities ($\chi^2 = 5.96$, $P = 0.04$) (Fig. 4). MP/IPs co-infection was not observed among HIV negative patients at the OPD of the hospital. Among PLWH, malaria/IPs co-infection was comparable among males (4.0%) and females (3.6%). On the other hand, the prevalence was significantly higher ($\chi^2 = 8.08$, $P = 0.04$) in those 21–30 years old (15.0%) than their counterparts as revealed in Table 5. Those who presented with fever had significantly higher prevalence ($P \leq 0.001$) of malaria/IPs co-infection (21.7%) than their afebrile counterparts (1.2%). While patients who attained viral load suppression had none significant ($P = 0.50$) lower prevalence (3.8%), those who have been on ART for more than three years showed a significantly lower ($P = 0.03$) prevalence of malaria/IPs co-infection (1.1%) than their respective counterpart as shown in Table 5.

Table 5
Malaria/IP co-infection prevalence among PLWH with respect to socio-demographic and clinical data

Categories	PLWH		Level of Significance (χ^2 , P)
	N	Malaria/IP positive % (n)	
Sex			
Male	50	4.0 (2)	$\chi^2 = 0.02$ P = 0.59
Female	140	3.6 (5)	
Age			
≤ 20	42	2.4 (1)	$\chi^2 = 8.08$ P = 0.04
21–30	20	15.0 (3)	
31–40	48	2.1 (1)	
≥ 40	80	2.5 (2)	
Fever status			
Febrile	23	21.7 (5)	$\chi^2 = 24.04$ P = 0.001
Afebrile	167	1.2 (2)	
Clinical signs			
Yes	39	2.6 (1)	$\chi^2 = 0.15$ P = 0.57
No	128	3.9 (5)	
Anaemic status			
Anaemic	146	4.1 (6)	$\chi^2 = 0.32$ P = 0.48
Non-anaemic	44	2.3 (1)	
Viral load (copies/ml)			
Not detected (< 50)	141	4.3 (6)	$\chi^2 = 0.28$ P = 0.50
≥ 50	41	2.4 (1)	
CD4 T-cell count (cells/μl)			
< 200	15	6.7(1)	$\chi^2 = 3.22$ P = 0.20
200–500	51	7.8(4)	
≥ 500	40	0.0(0)	
ART usage			
Yes	186	3.8(7)	$\chi^2 = 0.64$ P = 0.86
No	4	0.0(0)	
ART duration (years)			
≥ 1	36	11.1 (4)	$\chi^2 = 7.12$ P = 0.03

1–3	61	3.3 (2)	$\chi^2 = 0.38$ P = 0.45
≥ 3	89	1.1 (1)	
ART regimen First line	171	3.5 (6)	
Second line	15	6.7 (1)	

Haematological Abnormalities And Risk Factors Of Anaemia

Anaemia and leucopenia were found to be significantly higher ($P \leq 0.001$, $P \leq 0.001$ respectively) among PLWH (76.8% and 55.1% respectively) when compared with their HIV negative equivalent. The prevalence of thrombocytopenia was comparable among the different groups. PLWH had the lowest prevalence of microcytosis (43%, 80) and the difference was statistically significant ($\chi^2 = 45.94$, $P \leq 0.001$) as shown in Fig. 5.

As shown in Fig. 6, there was a significant difference ($P \leq 0.001$) when comparing haemoglobin values between the different infection categories; as those triple infected had the lowest haemoglobin value when compared with dual and single infected patients. A multinomial regression analysis was used to determine the factors associated with anaemia. As shown in Table 6, being 21–30 years old ($P = 0.029$), a male ($P < 0.001$), malaria parasite positive ($P < 0.001$), HIV positive ($P < 0.001$) and being febrile ($P < 0.001$) were significant risk factors of anaemia in the study population.

Table 6
Risk factors associated with anaemia in the study population

Parameters	Odds ratio (OR)	95% CI	P-value
^a Age group (years)			
≤ 20	0.792	0.499–1.257	0.32
21–30	0.571	0.346–0.944	0.03*
31–40	0.886	0.515–1.526	0.66
^b Male	2.601	1.779–3.802	∅ 0.001*
^c Febrile	0.330	0.186–0.585	∅ 0.001*
^d HIV positive	0.239	0.159–0.358	∅ 0.001*
^e MP positive	0.292	0.167–0.508	∅ 0.001*
^f MP/IPs co-infection	0.285	0.126–1.838	0.28
^g Helminth positive	0.591	0.221–1.580	0.29
^h Protozoan positive	0.825	0.456–1.492	0.52
ⁱ Leucopenia	0.573	0.387–0.848	0.01*
*Statistically significant P-value			
^a Age group: ≤ 20 years = 1; 21–30 years = 2; 30–40 years = 3; ≥ 41 years = 4			
^b Male = 1; Female = 2			
^c Febrile = 1; Afebrile = 2			
^d HIV positive = 1; HIV negative = 2			
^e MP positive = 1; MP negative = 2			
^f MP/IPs co-infection = 1; No MP/IPs co-infection = 2			
^g Helminth positive = 1; Helminth negative = 2			
^h Protozoan positive = 1; Protozoan negative = 2			
ⁱ Leucopenia = 1; No leucopenia = 2			

Discussion

Parasitic infections (especially malaria and intestinal parasites) are major public health concern in PLWH in developing countries, particularly Sub-Saharan Africa which has been reported to have the highest burden of HIV [2]. This study was a hospital- and community-based cross-sectional study carried out to determine the prevalence of malaria and intestinal parasites among PLWH and HIV negative individuals in Buea, in South West Region of Cameroon.

In the present study, the overall prevalence of malaria parasite was 16.6%, irrespective of the HIV status of the participants. This result is lower than the recently reported national prevalence (24.4%) [30]. This decrease could be attributed to the restless efforts of the Cameroon government over the years to implement malaria control strategies so as to curb down malaria morbidity and mortality on the entire territory; knowing that Cameroon comes fourth on the list of countries that account for the burden of malaria in the WHO African Region [5].

The prevalence of malaria parasite among PLWH was 16.4%, which is high compared to the prevalence reported in PLWH in other areas of the South West Region of Cameroon. This included the 7.8% reported in Limbe [10] and 2.3% in Buea [31]. It was also observed in this study that despite the efforts made to scale up insecticide-treated nets (ITNs) distribution so that universal coverage can be attained, coverage remains low and this could have contributed to this rise in malaria prevalence among PLWH.

The prevalence of malaria parasite was higher in HIV negative participants (21.3%) than in their HIV positive counterparts. This finding is in line with studies carried out in Cameroon [10] and Ethiopia [32] which also reported a higher malaria parasite prevalence in HIV negative participants at the OPD of the hospital when compared with their HIV positive counterparts. Although HIV positive patients are considered as a high-risk group for malaria, malaria is not the most frequent diagnosis in these patients. Majority of the HIV positive patients were under a combination therapy of ART and cotrimoxazole which probably protected them against infections. Cotrimoxazole has been reported in several studies to have some protective effect against parasitic infections, both in clinical trials [33, 34, 35] and in the hospital [22].

In this study, while MP prevalence was higher among HIV patients who did not attain viral suppression (viral load ≥ 50 copies/ml of blood), the majority of the HIV positive patients attained viral suppression due to their adherence to ART over the years. This is in line with recent findings in western Kenya [36] and further confirms the benefits of adherence to ART.

It is worth noting that HIV negative participants from the Buea communities had the least malaria parasite prevalence (14.8%) when compared with their counterparts. These malaria asymptomatic carriers are those who greatly fuel the transmission of malaria parasite in the community. This low prevalence shows that strategies implemented by the Cameroon government over the years are effective as this prevalence is lower than the reported national prevalence of malaria in 2018 [5].

Fever is one of the most common clinical sign and characteristic feature of both parasitic and microbial infections. In this study, febrile status of most of the participants irrespective of their study group was attributed to the presence of malaria parasite. This is in line with other studies carried out in Cameroon [10] and in Gabon [37] Also feverish PLWH had the highest prevalence of MP/IPs co-infection asserting the fact that fever remains an important feature in the clinical diagnosis of infections.

The prevalence of IPs in Cameroon varied from 27.8% in 2012 [38], to 14.6% in 2013 [39] and 13.0% in this present study. Considering the HIV status, PLWH had the highest prevalence of IPs (23.7%) when compared with their HIV negative counterparts. This prevalence among PLWH is lower than the 59.52% [39] and 57.48% [40] reported among HIV patients in the Centre and West Regions of Cameroon respectively. This observed decrease in the prevalence of IPs among HIV patients may be accredited to attainment of viral suppression by a majority as well as their better awareness of IPs and their causes. It may also be due to the improvement in care provided to PLWH by the government of Cameroon by ensuring a constant supply and provision of ART free of charge.

In line with other studies in Cameroon [15], Ethiopia [41] and in China [42], the prevalence of protozoa was higher than that of helminths. Both the HIV positive and HIV negatives were infected with *Cryptosporidium* spp; but PLWH had the highest prevalence of *Cryptosporidium* spp (16.8%). The prevalence of *Cryptosporidium* spp in this study is lower unlike the 44% reported earlier in Cameroon [15] and the 79.0%, in South Africa [43]. *Cryptosporidium* spp is recognized as an opportunistic parasite in HIV/AIDS patients because it tends to be present when the CD4 + T cell counts are below 200 cells/ μ L. Hence, this decreased prevalence among HIV patients could be attributed to the success of intervention efforts over the years in reducing the significance of opportunistic intestinal parasites in this at risk group. Hookworm was the most prevalent helminth among HIV patients with a prevalence of 2.6%. A prevalence of 2.53% and 2.7% have also been reported in studies carried out in Yaounde, [40] and in Buea [44] Cameroon.

This study revealed 3.7% of PLWH were co-infected with MP and IPs. Limited information is available on co-infection among PLWH in Cameroon; but when compared with other studies carried out among children [18, 45, 46], the prevalence is low. The mean age (35.5 ± 16.5 years) of PLWH as well as the undetectable viral load observed in the majority of these patients could account for this low prevalence. The lower the viral load, the faster the patient's immune system will recover, increasing the chances of fighting any infection easily. In addition, majority of this patients have been on ART regularly for more than a year which probably could have contributed in boosting their immune system against these parasites.

The overall prevalence of anaemia of 56.6% irrespective of their HIV status is higher than the 52.1% reported in previous study in the Mount Cameroon area [10]. This increase may be attributed to the rise in malaria parasite prevalence observed in the study when compared with previous studies in the area. Several authors have reported malaria parasite as a risk factor of anaemia [47, 48] due to the

pathogenesis of the infection that involves the destruction of both infected and non-infected red blood cells.

PLWH had a higher prevalence of anaemia (76.8%) when compared with their HIV negative counterparts. This prevalence is higher than the less than 56.0% reported in several studies carried out among PLWH [49, 9, 50, 51, 52, 53, 10]. The reason for the observed difference might be due to the heterogeneity of the study population as these previously reported anaemia prevalences were those of children or adults HIV patients, but this study included both children and adult participants. Malaria and HIV positivity, as well as fever were identified in this study as risk factors of anaemia and therefore contributed significantly to the high prevalence of anaemia. Mixed nutritional deficiencies (iron, folic acid, or vitamin B12), though not investigated in this study, could have contributed to this high prevalence of anaemia as reported by a study carried out by Volberding *et al.*, [54]. A direct comparison of prevalence of anaemia in different studies is difficult as the study population, inclusion and exclusion criteria and anaemia definitions were different.

Parasites like the malaria parasites and intestinal parasites have long been recognized as major contributors to reduced haemoglobin levels in endemic countries like Cameroon, thereby causing anaemia. In addition, PLWH on ART (containing Zidovudine) have been reported to influence the haemoglobin level in previous studies [55, 56, 57, 9, 10]. In this study, patients infected with both HIV, malaria and IPs had a significantly lower mean haemoglobin value (10.6 ± 1.21 g/dL). This in line with previous studies which reported though among children, low haemoglobin values in patients with co-infections when compared with those with single infections [58; 59].

The higher prevalence of Leucopenia observed among PLWH (55.5%) when compared with HIV negative counterparts has been reported in studies carried out in Ethiopia [60], Nigeria [61] as well as in Cameroon [50]. This high prevalence could be associated with profound immunodeficiency as more than half of PLWH had CD4 T cell count < 500 cells/ μ L of blood. While observation from this study revealed no significant difference in the prevalence of thrombocytopenia between PLWH and HIV negative participants, a decrease has been reported in other studies after ART introduction [62, 63]. Microcytosis was present among PLWH but the prevalence was lower (43%) when compared with their HIV negative counterparts. As PLWH attend an HIV specialty unit, they often receive medical talks with respect to their immunity, nutritional status and general wellbeing as part of the case management. This could have contributed to the low prevalence of microcytosis in this group which is often due to iron deficiency.

Even though different types of HIV and MP species have been reported in Cameroon, limited knowledge exist on the occurrence of MP/IPs co-infection among PLWH. Findings of this study fills some of the gaps although it has its limitations. Notwithstanding, the fact that ART-naïve HIV infected patients were not included in the study, nutritional deficiency as a cause of anaemia was not investigate, as well as the cross sectional nature of the study that provides a picture at a point in time, the findings are valuable to policy makers in guiding the management of these conditions. Future longitudinal studies on the impact of iron supplementation and vitamin B12 levels among PLWH may be of great relevance.

Conclusions

From this study, it can be concluded that malaria parasite and intestinal parasites (especially protozoa) remain a public health concern among PLWH with higher occurrences of both MP, IPs and MP/IP co-infections than in their HIV negative counterparts in the communities. Though a good majority of the HIV patients in the study had attained viral suppression, they are still more vulnerable to some of the parasites than HIV negative individuals and therefore can constitute a good source of contamination in their various communities. To achieve HIV epidemic control in 2021, the Cameroon government needs to intensify the implementation of the various strategies. Anaemia was the most prevalent haematological abnormality among HIV patients and gets exacerbated even with the viral load suppression in the population of PLWH. Hence, there is need for constant monitoring of the health status as well as nutritional status of this at risk group to better manage them.

Abbreviations

AIDS
Acquired immune deficiency syndrome;
ANOVA
Analysis of variance
ART
Anti-retroviral Therapy;
CAMPHIA
Cameroon population-based HIV impact assessment;
CD4 T
T-lymphocyte cell bearing CD4 receptors;
CI
Confidence interval
EDTA
Ethylene diaminetetraacetic acid
IPs
Intestinal parasites
IPI
Intestinal parasite infection
Hb
Haemoglobin
HIV
Human Immunodeficiency Virus
MCH
Mean corpuscular haemoglobin
MCHC

Mean corpuscular haemoglobin concentration

MCV

Mean corpuscular volume

MP

Malaria parasite

MPV

Mean platelet volume

OPD

Out Patient Department

P

Probability level

PLT

Platelets

PLWH

People Living With HIV

RBCs

Red Blood Cells

RDW

Red Cell Distribution Width

RHB

Regional Hospital Buea

SD

Standard Deviation

SPSS

Software Package of Social Sciences

UNAIDS

Joint United Nations Programme on HIV/AIDS

VL

Viral Load

WBC

White Blood Cell

WHO

World Health Organization

Declarations

Ethics approval and consent to participants

An administrative clearance was obtained from the South West Regional Delegation of Public Health, Buea, followed by an ethical clearance from the Institutional Review Board hosted by the Faculty of Health Sciences of the University of Buea (Reference Number: 661-09). Pre- and post-HIV counselling was

done by competent personnel. All test procedures were done free of charge with confidentiality maintained. Participation in the study was voluntary. Participants could ask questions and their decisions not to participate in the study were fully respected and this did not affect the services provided to them in any way. They were also free to withdraw from the study at any time that they wished. All information collected from the field remained strictly confidential.

Consent for publication

Not applicable

Availability of data and materials

All datasets on which the conclusions of the research rely are presented in this paper. However, data is available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

SMS conceived the study, designed and coordinated the field work, data collection and analysis as well as the write-up of the manuscript. IUNS participated in data analysis, supervision and revision of the manuscript. MMT was involved in data collection, interview of the participants and data analysis. HKK designed the study and revised the manuscript critically for important intellectual content. All authors read and approved the manuscript.

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Figures

Figure 1

Study flow diagram showing compliance of the participants.

Figure 2
Prevalence of malaria parasites (MP) among the different study groups

Figure 3

Prevalence of intestinal parasites (IPs) in the different study groups

Figure 4

Prevalence of MP/IPs co-infection in the study population

Figure 5

Prevalence of haematological abnormalities in the study population

Triple infection: Participants infected with HIV, malaria and intestinal parasites.
 Dual infection: Participants infected with any two (HIV/malaria, HIV/IPs, MP/IPs).

Figure 6

Box and whisker plots showing mean haemoglobin levels by infection categories.