



# **The Correlation of Intestinal Parasites and Malaria Coinfection on Hemoglobin Levels in People Living With HIV in Bamenda, Cameroon**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors NHN, AWT and LEA conceived and designed the experiments. Author AWT enrolled and performed the experiments. Authors AWT, JN and LEA contributed to data management, analysis, and interpretation. Authors AWT and JN wrote the manuscript, with authors NHN and LEA contributing to the scientific writing. Authors NHN and LEA reviewed the analyses and the final version of the manuscript. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Aims:** This study aimed to determine the presence of malaria and intestinal parasites (IP) and their association with anaemia among people living with HIV (PLWH) in Bamenda.

**Study Design:** This was a cross-sectional study that comprised PLWH on treatment.

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**Place and Duration of Study:** The study was conducted at St. Mary's Hospital and Azire Integrated Health Centre between February and July 2023.

**Methodology:** The study recruited 280 PLWH. Venous blood was collected to identify the malaria parasite and to measure haemoglobin levels, while stool samples were examined for the presence of IPs.

**Results:** The prevalence of malaria, IP, and their co-infection were 6.8%, 14.4%, and 3.6%, respectively. Malaria prevalence showed no relationship with sociodemographic characteristics ( $p \geq 0.05$ ). Only civil servants showed a significantly ( $p=0.033$ ) higher IP prevalence (19.0%) with respect to sociodemographic characteristics. A higher prevalence of malaria and IP co-infection was recorded in females, specifically in the 29-38 years age group (7.3%), married individuals (5.3%), those self-employed (4.5%), and those who had attained tertiary education (6.4%). However, only gender showed a significant difference ( $p = 0.013$ ). The prevalence of anaemia was 31.4% (88/280). Participants infected with Malaria, IPs, and Malaria/IPs coinfection were observed to have significantly ( $p \leq 0.05$ ) lower mean Hb levels. The risk factors of anaemia identified were: primary educational level [AOR=2.859,  $p=0.019$ ], helminths infection [AOR=0.128,  $p=0.000$ ] and unsuppressed viral load [AOR = 0.019,  $p=0.034$ ].

**Conclusions:** ART clinics should conduct periodic screening for anaemia and routine checkups for malaria and intestinal parasites among PLWH. Integrating malaria and IP control into HIV care can improve patient outcomes.

**Keywords:** Anaemia; HIV patients; intestinal parasites; malaria; prevalence; risk factors.

## 1. INTRODUCTION

Anaemia remains one of the major public health burdens affecting both developed and developing countries like Cameroon, making it a worldwide public health issue (Berhane *et al.*, 2020; Caldrex *et al.*, 2022; Kaudha *et al.*, 2023). Anaemia is common in people with the Human Immunodeficiency Virus (HIV) infection, with significant consequences on human health, economic and social growth (Bekolo *et al.*, 2023; Kaudha *et al.*, 2023; Tilahun *et al.*, 2024). The scaling up of antiretroviral therapy (ART) through the universal test and treat program has transformed HIV infection from a rapid killer to a manageable chronic disease (Mpaka-Mbatha *et al.*, 2022; Bekolo *et al.*, 2023). Though the use of ART has conferred a level of immunity to infections in persons living with HIV (PLWH), it has been reported to have adverse effects on the red blood cell indices of these patients (Sandie *et al.*, 2021)

The prevalence of anaemia varies among PLWH and ranges from 22.8% to 95% in different areas (Ageru *et al.*, 2019; Aemro *et al.*, 2022; Bayoï *et al.*, 2022; Ngwa *et al.*, 2024; Kaudha *et al.*, 2023). Several factors have been attributed to the cause of anaemia in HIV infection and these include; residence, gender, age, marital status, working status, educational status, opportunistic infections such as TB, nutritional deficiencies, low body mass index, and toxicities from

medications and suppression of bone marrow progenitors by HIV infected T cells, lower CD4 count, chronic disease and infections like malaria and intestinal parasites (Ageru *et al.*, 2019; Fentaw *et al.*, 2020; Caldrex *et al.*, 2022; Tilahun *et al.*, 2024). Intestinal parasites (IP) and malaria are among the most prevalent diseases in Sub-Saharan Africa, where they overlap extensively in their epidemiological distributions and frequently coinfect the same individuals (Sumbele *et al.*, 2021; Taheu *et al.*, 2021; Tilahun *et al.*, 2024).

There has been a continued spread of the malaria parasite despite global efforts to eradicate it. This was done by distributing free long-lasting insecticide-treated (bed) nets and prophylaxis. Complete eradication is not possible due to the presence of the Anopheles mosquito, which transmits the parasite (Sandie *et al.*, 2021). The control of these mosquitoes remains a major problem due to a lack of adequate sanitation, sewage treatment, and waste disposal in Cameroon

The burden of HIV and malaria in this region is one of the leading causes of morbidity and mortality (Sentongo *et al.*, 2020; Obegu *et al.*, 2024). It represents a challenging task, with thoughtful implications for haematocrit dynamics and health through various mechanisms. Malaria-induced anaemia is multifactorial and involves the release of proinflammatory

cytokines, such as tumour necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ), that is associated with the rupture of infected red blood cells (Obegu *et al.*, 2024).

In Cameroon, intestinal parasites are widespread due to the low availability of drinking water and other unhygienic factors (Takeu *et al.*, 2021; Mpaka-Mbatha *et al.*, 2022). Although there has been a decline in the global prevalence of these infections due to an increased number of funding bodies that have been contributing to the fight against these diseases in the last decade (Sandie *et al.*, 2021), this infection is still a serious health burden in people living with HIV. In Cameroon, the prevalence of intestinal parasites and HIV coinfection ranges from 11.0% -82.6% (Abange *et al.*, 2022; Bayoï *et al.*, 2022; Ntonifor *et al.*, 2022; Sandie *et al.*, 2022), while that of malaria and HIV coinfection ranges from 2.3% to 31.01 % (Mbah-Mbole *et al.*, 2022; Sandie *et al.*, 2022; Ngwa *et al.*, 2022). The complex interplay between these infections gives rise to haematological complications, making it difficult to effectively manage these diseases clinically.

Malaria parasites and intestinal parasitic coinfections have long been recognized as major contributors to reduced haemoglobin levels in HIV patients (Takeu *et al.*, 2021; Abange *et al.*, 2022; Sandie *et al.*, 2022; Mbah-Mbole *et al.*, 2022). While there is a wide variation in the prevalence of anaemia and its associated factors among PLWH in different studies in the country, there is an insufficiency of information on the prevalence and associated factors of anaemia among HIV/AIDS adult patients in the Northwest region that has a higher (4%) HIV prevalence than the national average (Tayong *et al.*, 2025).

To reduce the effects of anaemia in adults living with HIV, we need to be aware of the variables that may contribute to anaemia. The conditions regarding malaria and intestinal parasitic coinfection in the area, along with various control strategies such as the free availability of ART and cotrimoxazole, access to viral load testing, and community ART dispensation PLWH, require continuous follow-up. Therefore, this study aimed to assess the prevalence of malaria, IP, and malaria and IP coinfection, their combined impact on anaemia, and its associated factors among PLWH. The findings of this study are critical for guiding preventative, control, and treatment strategies to improve the health status of PLWH.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Duration

This was a cross-sectional study conducted between February and July 2023. The study comprised HIV patients on treatment at the St Mary Hospital and Azire Integrated Health Centre, Bamenda. Venous blood samples were collected and used to measure haemoglobin concentration and identify malaria parasites, while stool samples were collected to identify intestinal parasites.

### 2.2 The Study Area

This study was conducted at the Saint Mary Soledad Hospital, Alakuma and Azire Integrated Health Centres, which serve the HIV population of the Bamenda II subdivision and its environs. These Clinics provide antiretroviral therapy and care to HIV patients at no cost.

### 2.3 Study Population

The study population consisted of PLWH of all age groups and both sexes attending the Saint Mary Hospital (HIV/AIDS treatment centre) and Azire Integrated Health Centre.

### 2.4 Specimen Collection and Processing

Parents or guardians were instructed on how to collect a teaspoon of stool into sterile, leak-proof, wide-neck stool containers given to them. About 4 mL of venous blood was collected into EDTA tubes, anticoagulated by a trained lab technician. The blood samples were used to measure haemoglobin levels and prepare the thick and thin blood films for malaria microscopy observation.

### 2.5 Measurement of Haemoglobin Concentration (Hb)

The Hb level was determined using the Hb301 haemocue machine as per the manufacturer's instructions ([https://www.merkala.nl/media/bss/productattachment/Gebruikershandleiding\\_HemoCue\\_Hb\\_301\\_meter.pdf](https://www.merkala.nl/media/bss/productattachment/Gebruikershandleiding_HemoCue_Hb_301_meter.pdf)) and the values recorded. Hb values were used to classify anaemia based on age and sex as follows; mild anaemia; Hb 10 to 12g/dl in women and 10 to 13.0g/dl in men, moderate anaemia; Hb 8 to

9.9g/dl in both sexes, severe anaemia; Hb 6.5 to 7.9g/dl in both sexes and life-threatening anaemia; Hb<6.5g/dl (Calder *et al.*, 2022).

## 2.6 Parasitological Analysis

**Detection of Malaria Parasites:** Approximately three drops of blood were used to prepare a thick film on labelled clean slides, the films were dried for about 2-3 minutes, stained with 10% Giemsa solution, and then washed after 10 minutes using clean water. The slides were allowed to air dry, and a drop of immersion oil was added to the slide, which was then examined microscopically for malaria parasites using a 100X objective lens. If parasites were observed, the density was determined by counting the number of parasites against 200 leucocytes. The parasite density was calculated by dividing the number of parasites by 200 and then multiplying the result by the patient's actual white blood cell count.

### Identification of Intestinal Parasites Using the Formol-Ether Concentration Technique:

Wet mounts preparation of stool and parasite identification was done as described by Ntonifor *et al* (2020). In brief, a small portion of the stool sample was emulsified in physiologic saline (0.85% NaCl solution) on a clean glass slide, covered with a coverslip, and observed under a light microscope. For the formol-ether concentration method, using an applicator stick, approximately 0.2 grams of stool was emulsified in 7 ml of 10 % formalin in a mortar. A surgical gauze was used to filter the stool sample into a centrifuge tube. 3 mL of diethyl ether was added to the solution in the centrifuge tube. The tubes were thoroughly shaken for 30 seconds, then centrifuged at 3000 rpm for 3 minutes. The supernatants were discarded, and the deposit in the tube was placed on a slide. A drop of Lugol's iodine solution was then added to it. The slide was then covered with a cover slip and examined under the microscope at 10X objective before changing the lens to 40X. The eggs and cysts observed were counted and recorded. The number of eggs or cysts was counted for each species of the parasite in the entire preparation and reported as the number of eggs per gram of faeces. An attempt was made to go through all the prepared fields before a sample was reported negative for parasites.

## 2.7 Modified Ziehl-Neelsen Staining Technique

Thin smears were prepared using sediment obtained by the formol-ether concentration technique. The slides were allowed to air dry and fixed with methanol for 3 minutes. Then, the smears were flooded with carbon fuchsin for 15 minutes, washed with tap water, discoloured with 1% acid-alcohol for 15 seconds, rinsed in tap water, flooded with 0.5% methylene blue for 30 seconds, washed with water, allowed to dry, and observed under a light microscope with 100X objective.

## 2.8 Statistical Analysis

The collected data were entered into an Excel spreadsheet and analyzed using SPSS version 23 software (IBM SPSS Inc., Chicago, IL, USA). Descriptive analysis for clinical parameters was presented as numbers (n) and percentages (%). The Pearson chi-square test was used to compare the prevalence of parameters between groups and determine whether the data differed significantly from what was expected. The T-test was used to test differences between the means of the two groups. Univariate and multivariate analysis were used to identify the risk factors. Statistical significance was set at a p-value of  $\leq 0.05$ .

## 3. RESULTS

### 3.1 Sociodemographic and Baseline Clinical Characteristics of the Study Population

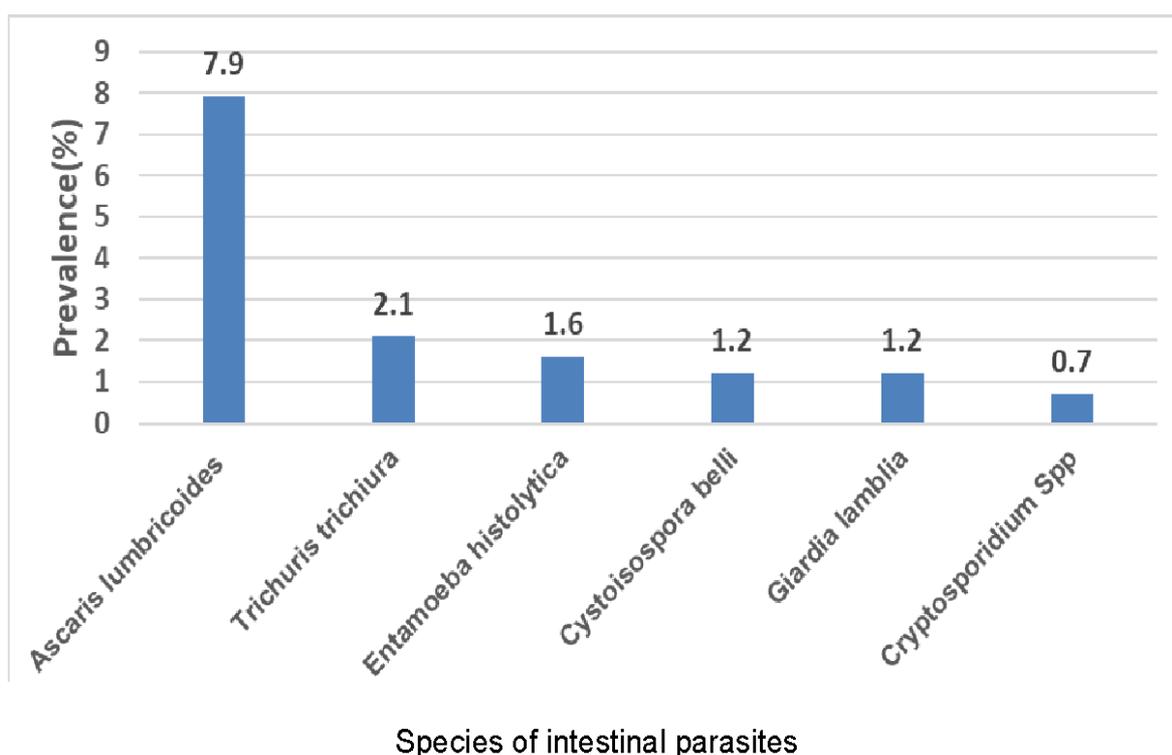
A total of 280 participants aged between 16 and 62 years took part in the study. The majority (62.9%; 176/280) of the participants were female, in the age range of 29-38years (48.9%; 137/200), married (60.7%; 170/280), self-employed (63.2%; 177/280) and had not been to school (28.9%; 81/280) as shown on Table 1. Clinically, more than half of the participants had a suppressed viral load (87.9%; 246/280) and were receiving first-line antiretroviral therapy (ART) treatment (87.5%; 245/280).

### 3.2 Prevalence of Malaria

A total of 19 (6.8%) participants were positive for malaria. The prevalence of malaria was higher in females (6.8 %; 12/176), participants in the age

**Table 1. Sociodemographic and baseline clinical characteristics of the study population (n=280)**

Parameters	Variables	Frequency	Percentages
Gender	Female	176	62.9
	Male	104	37.1
The age range in years	<18	26	9.3
	18-28	82	29.3
	29-38	137	48.9
	>38	35	12.5
Marital status	Divorced	15	5.4
	Married	170	60.7
	Single	95	33.9
Level of education	None	81	28.9
	Primary	59	21.1
	Secondary	62	22.1
	Tertiary	78	27.9
Occupation	Civil servant	58	20.7
	Self-employed	177	63.2
	Unemployed	45	16.1
Viral load	Suppressed (detected but $\leq$ 1000 copies/mL)	246	87.9
	Unsuppressed (above 1000 copies/mL)	34	12.1
Type of treatment	First line	245	87.5
	Second line	35	12.5



**Fig. 1. Prevalence of the different gastrointestinal parasitic species within the study population**

**Table 2. Prevalence of malaria with respect to socio-demographic parameters (n=280)**

Variables	Options	Number examined	Number infected (%)	χ <sup>2</sup>	p-value
Gender	Male	104	7(6.7)	0.00	0.978
	Female	176	12(6.8)		
Age group in years	<18	26	2(7.7)	1.29	0.730
	18-28	82	7(8.5)		
	29-38	137	9(6.6)		
	>38	35	1(2.9)		
Level of education	None	81	7(8.6)	1.78	0.618
	Primary	59	5(8.5)		
	Secondary	62	4(6.5)		
Occupation	Civil servant	58	3(5.2)	2.5	0.284
	Self-employed	177	15(8.5)		
	Unemployed	45	1(2.2)		
Marital status	Divorced	15	1(6.7)	3.09	0.213
	Married	170	15(8.8)		
	Single	95	3(3.2)		
	Tertiary	78	3(3.8)		

**Table 3. Prevalence of intestinal parasites concerning socio-demographic parameters (n=280)**

Variables	Options	Number examined	Number infected (%)	χ <sup>2</sup>	P-value
Gender	Male	104	12(11.5)	0.257	0.612
	Female	176	24(13.6)		
Age group in years	<18	26	4(15.4)	4.784	0.188
	18-28	82	9(11.0)		
	29-38	137	22(16.1)		
	>38	35	1(2.9)		
Marital status	Divorced	15	0(00.0)	2.459	0.293
	Married	170	24(14.1)		
	Single	95	12(12.6)		
Occupation	Civil servant	58	11(19.0)	10.503	0.033
	Self-employed	177	22(12.4)		
	Unemployed	45	3(6.7)		
Level of education	None	81	13(16.0)	3.500	0.174
	Primary	59	6(10.2)		
	Secondary	62	5(8.1)		
	Tertiary	78	12(15.4)		

group 18-28years (8.5%; 7/82), those who had attained primary education (8.6%; 7/81) self-employed participants (8.5%; 15/177), and those that were married (8.8%; 15/170). However, these differences were not significant ( $p < 0.05$ ), Table 2.

The overall prevalence of IPs was 12.9% (36/280). The most common IP identified was

*Ascaris lumbricoides* (7.9%; 22/280), and the least was *Cryptosporidium* Sp (0.7%; 2/280) (Fig. 1). Multi-helminthic infections were observed in 1.4% (4/280) of the participants, with *A. lumbricoides* and *T. trichiura* being the most occurring multi-infections (0.7%; 2/280), while both *A. lumbricoides* and *E. histolytica* and *A. lumbricoides* and *T. trichiura* recorded a case (0.4%; 1/280).

In Table 3, the gender-based prevalence of IP showed that females (13.6%; 24/176) were the most affected, although the difference was not statistically significant (P = 0.621). Similarly, parasite prevalence was highest (16.1%; 22/137) among individuals aged 29-38 years, those who were married (14.1%; 24/170), and those who had attained primary education (16.0%; 13/81). These differences were not significant (p<0.05). On the contrary, only civil servants showed a significantly (p=0.033) higher prevalence (19.0%; 11/58).

The prevalence of malaria and IP co-infection was found to be 3.6 % (10/280). Except for gender showing a significantly (p=0.013) higher prevalence in males (5.7 %; 10/1104), all other sociodemographic characteristics did not show any significant difference (p> 0.05) between malaria and IP coinfection. However, a higher malaria and IP co-infection prevalence was recorded in was high in the age group 29-38years (4.4%; 6/137), those who were

married (5.3%; 9/170), participants who were self-employed (4.5%; 8/177), and those who had attain tertiary education (6.4 %; 5/78) Table 3.

### 3.3 Prevalence of Anaemia

The Hb level ranged from 7.3-18.2 g/dL, with a mean (±SD) of 13.62 (±2.28) g/dL. In Fig. 2, the prevalence of anaemia was 31.4% (88 /280). Of these, most participants (86.36%; 76/88) had mild anaemia (Fig. 3).

### 3.4 Effect of Parasite on Hb (g/dL) Level

In Table 5, participants infected with Malaria, IPs, and Malaria/IPs coinfection were observed to have a lower mean HB(g/dL) compared with their negative counterparts. Except for *Plasmodium* spp., there was no significant difference (p = 0.053). Haemoglobin levels were statistically significant with IP (p=0.000), and Malaria/IPs coinfections (p=0.000).

**Table 4. Prevalence of malaria and IPI coinfection concerning socio-demographics and Characteristics (n=280)**

Characteristic	Variables	Number examined	Number infected	χ <sup>2</sup>	P- value
Gender	Male	104	0(0.0)	6.128	0.013
	Female	176	10(5.7)		
Age group in years	<18	26	1(3.8)	1.564	0.668
	18-28	82	3(3.7)		
	29-38	137	6(4.4)		
	>38	35	0(0.0)		
Marital status	Divorced	15	0(0.0)	3.771	0.152
	Married	170	9(5.3)		
	Single	95	1(1.1)		
Occupation	Civil servant	58	1(1.7)	1.275	0.529
	Self-employed	177	8(4.5)		
	Unemployment	45	1(2.2)		
Level of education	None	81	2(2.5)	2.807	0.422
	Primary	59	2(3.4)		
	Secondary	62	1(1.6)		
	Tertiary	78	5(6.4)		

**Table 5. Effect of parasitic infection on haemoglobin level**

Variable	Mean ±SD Hb concentration g/dL		T- value	P value
	Negative	Positive		
Malaria	13.69±2.19	12.65±3.08	1.941	0.053
IP	13.85±2.18	12.04±2.31	4.629	0.000
Malaria and IP coinfection	13.74±2.17	10.43±2.62	4.681	0.000

### 3.5 Factors Associated with Anaemia in the Study Participants

In univariate analysis (Table 6), participants with any of the IP had a significantly higher prevalence of anaemia [Crude odds ratio (COR) = 0.28, 95% confidence interval (CI) = 0.128-0.347;  $p < 0.000$ ]. Although participants who did not attend school [COR=0.36, 95% CI=0.121- 1.079;  $p=0.068$ ] and those with unsuppressed viral load [COR=0.455, 95% CI=0.194- 1.064;  $p=0.068$ ] had a higher prevalence, this trend was not statistically significant. Variables in the univariate analysis that had  $p < 0.25$  were considered for multivariate analysis. In the univariate analysis, the risk factors for anaemia at a 95% CI showed a trend for participants who did not attend school, with a ratio of 0.362 (0.121-1.079).

After controlling for educational level with other variables in multivariate analysis, the risk was 2.859 times higher [Adjusted odd ratio {AOR}=2.859, 95%CI=1.193- 6.854;  $p=0.019$ ]. Also, this data revealed that HIV patients who tested positive for any of the helminths had a higher risk of developing anaemia [COR=2.28, 95%CI=0.128-0.347;  $p=0.000$ ] in univariate analysis. The risk of developing anaemia increased by 10.619 % (95% CI=.472-25.218;  $p=0.001$ ), after adjusting this parameter with the rest of the variables in the multivariate model. Viral load data showed a trend in participants with unsuppressed viral load [COR=50.94, 95%CI=0.194- 1.064;  $p=0.069$ ]. After controlling for other variables in multivariate analysis, the risk increased by 2.45 times [AOR = 0.019, 95% CI = 1.072-5,605,  $p = 0.034$ ].

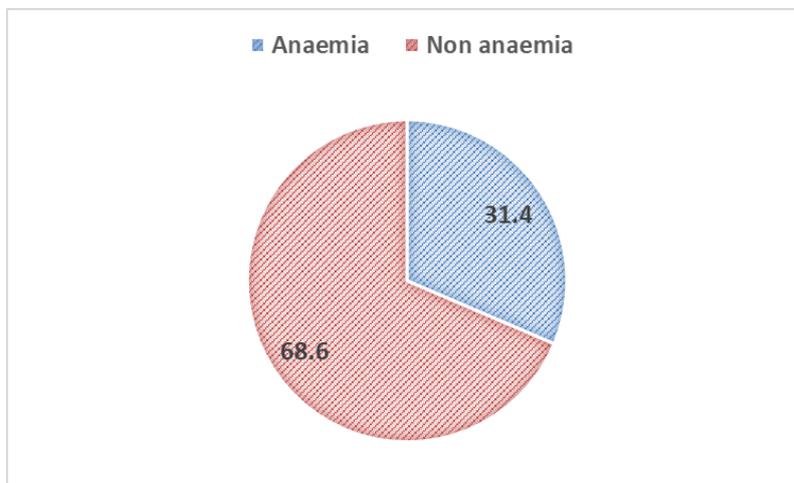


Fig. 2. Prevalence (%) of anaemia in the study population

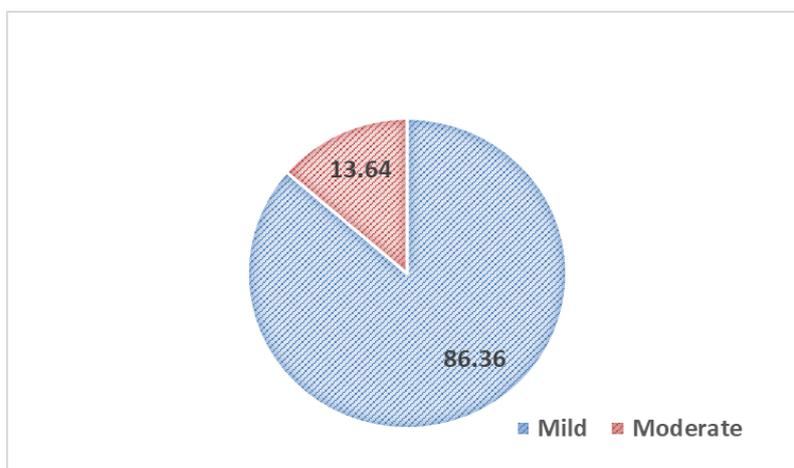


Fig. 3. Type of anaemia in the study population

**Table 6. Analysis of related risk factors for anaemia in HIV in Bamenda, Cameroon (n=280)**

Variables	Category	Frequency n (%)	Positive for Anaemia n (%)	COR	Odds ratio (95% CI) p-value	AOR	p-value
Age (years)	<18	26	9(34.6)	1.185(0.326-4.300)	0.797		
	18-28	82	25(30.5)	0.954(0.328-2.776)	0.931		
	29-38	137	47(34.3)	1.039(0.377-2.865)	0.941		
	>38	35	7(20.0)	Ref			
Gender	F	104	29(27.9)	1.044(0.566-1.923)	0.891		
	M	176	59(33.5)	Ref			
Marital status	Divorced	15	5(33.3)	0.994(0.286-3.461)	0.993	0.941(0.282-2.962)	0.881
	Married	170	62(36.5)	0.424(0.113-1.595)	0.205	2.130(0.611-7.433)	0.236
	Single	95	21(22.1)	Ref		Ref	
Occupation	Civil servant	58	25(43.1)	0.971(0.340-2.774)	0.956		
	Self-employed	177	48(27.1)	0.712(0.305-1.660)	0.432		
	Student	45	15(33.3)	Ref			
Educational level	None	81	33(40.7)	0.362(0.121-1.079)	0.068	2.859(1.193-6.854)	0.019
	Primary	59	11(18.6)	0.636(0.251-1.612)	0.340	1.562(0.746-3.270)	0.237
	Secondary	62	21(33.9)	0.856(0.360-2.035)	0.725	1.015(0.477-2.162)	0.968
	Tertiary	78	23(29.5)	Ref		Ref	
Treatment	First	245	79(32.2)	0.636(0.254-1.591)	0.333		
	Second	35	9(25.7)	Ref			
BMI grouping	Normal	125	39(31.2)	1.219(0.384-3.873)	0.737		
	Obese	27	10(37.0)	1.121(0.362-3.466)	0.843		
	Overweight	75	23(30.7)	1.554(0.539-4.480)	0.414		
	Underweight	53	16(30.2)	Ref		Ref	
Helminth	No	244	61(25.0)	2.28(0.128-0.347)	0.000	10.619(4.472-25.218)*	0.000
	Yes	36	27(75.0)	Ref		Ref	
MP	Negative	261	79(30.3)	5.699E8(0.000)	0.999		
	Positive	19	9(47.4)	Ref		Ref	
Co-infection	No	270	79(29.3)	0.000(0.000)	0.999		
	Yes	10	9(90.0)	Ref		Ref	
Viral load	suppressed(<1000copies/mL)	246	72(29.3)	0.455(0.194-1.064)	0.069	2.451(1.072-5605)*	0.034
	unsuppressed(>1000copies/mL)	34	16(47.1)	Ref		Ref	

Note: p < 0.05 was taken as significant- Ref stands for the reference category

#### 4. DISCUSSION

Intestinal parasites and malaria are major public health concerns among PLWH in developing countries, especially Sub-Saharan Africa, which has been reported to have the highest burden of HIV (Sandie et al., 2021). This study aimed to identify the risk factors associated with anaemia among HIV-infected individuals in the Bamenda II Subdivision, Cameroon.

In our present study, the overall prevalence of the malaria parasite was 6.8%. However, it was lower compared to the 24.4%-36.4% prevalence reported in similar studies carried out in the same region (Bayio et al., 2021; Sandie et al., 2021; Ntonifor et al., 2023) and the 22.9%-27.7 % range reported elsewhere (Jegade et al., 2017; Gumel et al., 2021). This low prevalence may be related to the policies implemented by the government over the years to control malaria, aiming to flatten the curve of malaria morbidity and mortality throughout Cameroon. This includes the free distribution of long-lasting insecticide-treated bed nets and the use of trimethoprim-sulfamethoxazole prophylaxis in Cameroon in 2016 (Bayio et al., 2021; Sandie et al., 2021; Fokam et al., 2017). This decrease in malaria prevalence could also be a result of the fact that most HIV patients know that they are immunocompromised and, as such, carry out preventive measures.

On the other hand, the prevalence of malaria parasites among HIV patients in this study was higher compared to the 7.3-2.3% % prevalence range reported in HIV patients in Cameroon and elsewhere (Sandie et al., 2019; Ssentongo et al., 2020).

Intestinal parasites recorded a low prevalence of 12.9% in this study. This prevalence falls within the 11.4% - 14.4% range reported in countries (Ketema et al., 2017; Sandie et al., 2021) and is lower compared to the 18%- 82.6% recorded elsewhere (Taheu et al., 2021; Mpaka-Mbatha et al., 2022). This prevalence may be associated with the fact that people living with HIV (PLWH) have a compromised immune system. It may also be due to the result of the poor socioeconomic and socio-political crisis that have led to a decrease in hygienic and sanitary measures (little or no access to good drinking water, good toilets, overcrowding in homes) by individual since these participants are mostly internally displaced persons from other parts of the Northwest region living in precarious conditions.

Parasites identified were *Ascaris lumbricoides*, *Trichuris trichiura*, *Entamoeba histolytica*, *Cystoisospora belli*, *Giardia lamblia*, and *Cryptosporidium* spp. Most of the recovered parasites were opportunistic infections that take advantage of the immunocompromised state of the patients' immune system. Varying types of parasites have been identified in PLWH, including *Blastocystis hominis*, *Microsporidium* spp., and *Sarcocystis* sp. *Isospora belli*, *Hymenolepis nana*, *Strongyloides stercoralis*, *Cyclospora cayentensis*, *Schistosoma mansoni*, hookworms, and *Taenia* spp as reported in other studies (Rodríguez-Pérez et al., 2019; Sandie et al., 2021; Taheu et al., 2021; Ntonifor et al., 2022). The differences in the type of intestinal parasites could be attributed to variations in study design and differences in socio-economic and environmental conditions.

In this study, the prevalence of MP and IP co-infection was 3.6%. Although the information available on IPs and MP co-infection among PLWH in Cameroon is limited, the prevalence is low when compared to studies carried out elsewhere (Angora et al., 2019; Sandie et al., 2021). This low prevalence is credited to the same factors responsible for the low prevalence of IPs and malaria as outlined above.

The impact of intestinal parasites and malaria on the haemoglobin levels in HIV patients cannot be underestimated. In this study, patients infected with HIV, malaria, and IPs had lower mean haemoglobin values. The high Hb concentration seen in malaria-negative patients has been reported elsewhere (Obeagu et al., 2024). *Plasmodium* spp. triggers the release of pro-inflammatory cytokines, such as tumour necrosis factor-alpha and interleukin-1 $\beta$  (IL-1 $\beta$ ), contributing to anaemia. Inflammatory cytokines targeting infected red blood cells have been shown to decrease haematocrit levels. Secondly, HIV and malaria-induced immunomodulatory effects contribute to dysregulated erythropoiesis through direct destruction of erythrocytes, sequestration of infected and uninfected erythrocytes by the spleen, and decreased erythropoiesis (Ssentongo et al., 2020; Obeagu et al., 2024). IP feeds on host tissues, including blood, and interferes with the absorption of nutrients like iron and B12, leading to iron deficiency and potentially chronic intestinal blood loss. IP feeds on host tissues, including blood, and interferes with the absorption of nutrients like iron and B12, leading to iron deficiency and potentially chronic intestinal blood loss. It has

been reported that *A. lumbricoides* and *T. trichiura* are strongly associated with low serum ferritin levels, leading to intestinal blood losses (Rabui et al., 2024; Mpaka-Mbatha et al., 2022).

Anaemia prevalence was 31.4%, with most of the anaemic participants (86.36%) presenting with mild anaemia. This value was lower than 40%-93.3% (Bate et al., 2016; Ezechi et al., 2019; Bayoï et al., 2022; Tilahun et al., 2024; Frederick et al., 2024) observed in similar studies in other towns in Cameroon reported similar cases of higher mild anaemia to moderate anaemia (Ezechi et al., 2019; Tilahun et al., 2024). However, it was higher compared to the 13.3%-16.8% recorded in other studies elsewhere (Fentaw et al., 2020; Kaudha et al., 2023). Anaemia in PLWH may occur due to a deficit in cytokine production with subsequent effects on haematopoiesis, decreased erythropoietin concentrations, vitamin B12 deficiency, autoimmune destruction of red blood cells, opportunistic infectious agents, such as *Mycobacterium avium* complex, parvovirus, use of chemotherapeutic agents such as HAART, and trimethoprim-sulfamethoxazole (Sullivan et al., 1998; Bate et al., 2016; Ezechi et al., 2019; Berhane et al., 2020; Bayio et al., 2022; Obegu et al., 2024). The disparity in these studies could be a result of discrepancies in socio-demographic characteristics, the type of HAARTs used, differences in sample size, and food disparities.

No formal education, presence of helminth infection, and unsuppressed viral load were identified as risk factors for anaemia among PLWH. Participants with no formal education recorded the highest prevalence because of a lack of adequate knowledge of infection prevention and nutritional dieting, which may lead to anaemia. As such, there is a need to scale up health educational talks on the implementation of anaemia control strategies among the population. This can be supported by the fact that malaria and IP infections were highest in people who had no formal education. This observation aligns with other studies conducted elsewhere (Miressa et al., 2021; Kaudha et al., 2023; Tilahun et al., 2024).

Parasitic infections were strongly associated with anaemia, indicating that HIV-infected individuals with helminths were over ten times more likely to be anaemic than those without such infections. This aligns with existing literature suggesting that helminths exacerbate anaemia through chronic

blood loss, nutritional deficiencies, and immune modulation, particularly in immunocompromised individuals (Rodríguez-Pérez et al., 2019; Means et al., 2020).

Viral load count showed significant associations with anaemia. Participants with unsuppressed viral loads recorded a higher prevalence of anaemia. Although a good majority of the HIV patients in the study had attained viral suppression, they were still more vulnerable to some of the parasites. The suppression of viral replication and restoration of immune function contribute to the amelioration of anaemia (Bate et al., 2016). The high prevalence observed in individuals with unsuppressed viral loads requires further investigation, such as testing for the presence of the resistant ART gene. This result is similar to findings elsewhere (Sullivan et al., 1998; Kaudha et al., 2023; Obegu et al., 2024) and contrary to studies by Ezechi et al. (2019), who stated that viral load was not associated with anaemia.

Although other variables such as age group, gender, marital status, occupation, type of treatment, BMI, Malaria parasite, and malaria and intestinal parasitic coinfection showed varying degrees of association with anaemia in the bivariate analysis, they were not statistically significant in the adjusted multivariate model. This finding contradicts other studies that have identified these factors as risk factors. Some studies have reported male sex, younger age groups, drug regimen, divorced marital status, presence of malaria, and malaria and intestinal parasitic coinfection, and increased age as risk factors for anaemia. (Aemro et al., 2022; Kaudha et al., 2023). This discrepancy in results could be attributed to the difference in geographical location, study design, and the number of years the study participants have lived with the virus (Rodríguez-Pérez et al., 2019).

Although malaria infection and coinfections showed a significant decrease in Hb levels in positive participants, they were not identified as risk factors, contrary to reports in other studies (Ageru et al., 2019; Ssentongo et al., 2020).

The prevalence of malaria and its coinfection with intestinal parasites was low to allow meaningful analysis of their association with haemoglobin concentration, indicating that the aetiology of anaemia is usually multifactorial (Sanyaolu et al., 2019). The prevalence of malaria and its coinfection with intestinal parasites was low, allowing for a meaningful

analysis of their association with haemoglobin concentration. This indicates that the etiology of anaemia is usually multifactorial (Sanyaolu *et al.*, 2019). Furthermore, the use of ART by these patients may have reduced the frequency of malaria episodes. A contrary result was reported in sub-Saharan Africa, which observed that malaria was a major contributor to anaemia (Sanyaolu *et al.*, 2019; Ageru *et al.*, 2019).

## 5. CONCLUSION

The prevalence of anaemia among these study participants is high; this might affect the treatment outcome, increasing morbidity and mortality of the participants. Public health interventions targeting individuals with no formal education, intestinal parasites, and unsuppressed viral loads will improve the health-care of PLWH. There is a need, therefore, to implement malaria and intestinal parasitic screening as part of routine care in PLWH.

## ETHICAL APPROVAL AND CONSENT

The study protocol was approved by the University of Bamenda Institutional Review Board, Cameroon (2023/0875H/UBS/IRB). Participation was voluntary after the objectives of the study were explained to all the participants (parents of children). Parents or guardians of children under 18 years signed minor consent forms on behalf of their children.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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