Malaria and intestinal coccidian parasites co-infections in adult patients in the Fundong Health District, Northwest Region, Cameroon.

Abstract

Background:Malaria and intestinal coccidian parasites are both protozoan and their interaction in co-infected patients is still not clear. Patients infected with both malaria and coccidian parasites experience diarrhoea, gastro-intestinal and health problemsthat maybe life threating. We studied intestinal coccidian parasites (OIPs) andthe human immunodeficiency virus (HIV)in adult patients with malaria in Fundong Health District, Northwest of Cameroon.

Method:A prospective cross-sectional study carried out between April and December 2022. Malaria patients were identified by the presence of the *Plasmodium* parasite in Giemsa blood-stained films. HIV status was determined using the rapid diagnostic test (RDT). Stool samples were subjected to wet preparation and formol-ether concentration technique to detect intestinal coccidian parasites. Pearson's Chi-Square (χ^2) and binary logistic regression were performed as part of the statistical analysis.Statistical significance was set a P-value<0.05.

Results:**Results**: Three hundred and thirty (330) patients took part in the study with mean age 37.02(±15.235) years. Malaria co-infection prevalence with coccidian parasites was 19.4%., with other gastro-intestinal parasites was 5.5%., and with HIV was 8.2%. Fever was most reported (95%), abdominal pains (26%), anddiarrhoea (11%). Fever, and abdominal pain were significantly associated with simple malaria (*P*value= 0.015) and severe malaria (*P*value= 0.004) as well as the HIV (*P*value= 0.003). The odds of having HIV 2.6(95% CI: 0.095-71.016) *P*value=0.571) times higher with fever compared to abdominal pain 0.636 (95% CI: 0.124-3.259 *P*value=0.587) and diarrhoea 0.153 (95% CI: 0.013-1.772, *P*value=0.133). The odds of severe malaria was 3.5(95% C.I: 1.2-10.4) times higher with fever compared to those with abdominal pain 0.4(95% C.I: 0.2-0.8). living with a child <2 years, washing without using soap predicted for coccidian infection accounting for 23.6% of the variance in the model.

Conclusion:Malaria co-infection withintestinalcoccidian parasites and HIV have been neglected in the FundongHealth District. Screening suspected malaria patients for intestinal coccidian and HIV is recommended and a combined control and prevention strategy needs to be considered.

Keywords: Malaria, intestinal coccidian parasite, HIV co-infection, prevalence, screening.

Introduction

Malaria and intestinal parasitesare parasitic diseases and are highly endemic in the tropics especially in Sub-Sahara Africa(SAA), and in impoverished communities, where hygiene and sanitation conditions are below standards. Over the past years malaria has witnessed a general decline in many countries,though a significant number of people still die of the disease especially children[1]. Globally, in 2021, there were 249 million cases reported and 619,000 million deaths were registered, with 234 million cases and 593,000 deaths reported in the Africa region alone.This accounts for 95% of all global malaria cases and 96% of all deaths [2]. Malaria is caused by a species of protozoans called *Plasmodium species*, with *P. falciparum*being most virulent, and accounting for a majority of malaria cases [3]. Cameroon lies within the endemic area of high malaria transmission, whereby everyone is at risk of malariainfection [4]. In 2021 over 6.6 million cases of malaria were reported with a slight witnessed in an increase due to the Coronavirus 2019 (COVID-19)pandemic [3].

Morbidity and mortality due to malaria and intestinal parasites can be controlledthroughChemotherapy, primary prevention by distribution of insecticide treated bed-nets (ITNs), indoor residual sprays (IRS) and the national deworming programme to control intestinal parasites. However, routine deworming is not effective in treating opportunistic intestinal parasitic diseases [5], making its control one of the future public health interventions priorities.Low-income countries still bear the greatest burden of intestinal parasitic infections [6].

Conditions favouring the transmission of malaria and intestinal parasites are similar, thus facilitating multiple infections to occur.Among intestinal parasitic diseases occurring in coinfection with malaria are opportunistic intestinal parasitic diseases caused by intestinal coccidian parasites.Among the pathogenic coccidian parasites are *Cryptosporidium*, *Isospora*, *Cyclospora*, *Microsporidia*, *Blastocystis*, and *Toxoplasma gondi*. *Plasmodium* species and intestinal coccidian parasites are protozoans [7]. Immuno-compromised Individuals such as HIV and AIDs, childrenand people with underlying medical conditionsbeing the primary targets [1, 6-7]. Various studies carried out in Africa on opportunistic intestinal parasites have been most focused on HIV patients, and on their prevalence in children [8-9]. Characteristics favouring malaria transmission are similar to those favouring the transmission of intestinal parasitic infections (IPIs).Where these parasites exist, the vulnerable population are often exposed to multiple infectionsof malaria and IPIs resulting in adverse health consequences ranging from mild to life threating conditions [6]. Individuals faced with these conditionsincluding HIV and AIDs,have an increased risk of developing severe haematological abnormalities andsometimes leading to death [1, 6, 10].Although treatment for malaria using Artemisinin combination therapy (ACT), is largely available in Cameroon, however the treatment of the opportunistic intestinalcoccidian infections are unavailable. Thismakes the health system vulnerable,in case an outbreak occurs they will eventually become overwhelmed. Such outbreaks have been reported in many other countries including the USA, and Italywhere water, foods and animals have been identified as their major routes of transmission [11-12].

The situation in Cameroon regarding malaria-intestinal coccidian parasitic diseases is unknown as well as data related to morbidity and mortality because of neglect. Thisstudy was undertaken to determine the prevalence of malaria-intestinal coccidian parasitic diseases in the Fundong Health District (FHD) and the associated risk factors. The data will provide relevant information to policymakers in the area and in the country.

Methods and materials

Study site

Fundong Health District is located between latitude 6° 4'and 6°23' to the North of the equator and longitude 10° and 10° 33' to the East of the Greenwich Meridian and its attitude ranges from (800-2500m) above sea level[13]. The FHD is located, 80Km Northwest of the Regional Capital city of Bamenda, has different levels of urbanization with vast majority of the settlements typically rural (80%) with a population of approximately 250,000 inhabitants. The FHD is located within the malaria high risk regions in Cameroon with moderate levels of transmission during and just after the rainy season which begins in March and ends in Octobers[4]. Malariaprevalence in the region is estimated at roughly over 10%[14-16]. Its tropical climate makes it a favourable environment for the malaria vector to thrive. Most of the Health District is rural and majority of the population is poor, also creates a favourable condition for IPIs that have been described as diseases of the poor [17] to also thrive. Due to inadequate access to safe drinking water, the prevailing poor hygiene and sanitation and low socio-economic status of the inhabitants, that exist in the region and Cameroon as whole, they expose the population to the risk of water borne infections and opportunistic parasitic diseases in particular[6, 18].

Study population

Only adult malaria patients who signed the informed consent and willingly accepted to be tested for opportunistic intestinal parasites were recruited as participants for the study. Those who had been on antibiotics and anti-parasitic drugs two weeks prior to consultation were excluded from the study.

Specimen collection and processing

Thesigningof the consent form by participants was followed byhealth education on the purpose of the study and instructions on how tocollect stool. Collect a teaspoon full sample into a labelled sterile stool container. About 4mL of whole blood was collected into an EDTA anti-coagulated tube to perform full blood count (FBC). Thick and thin films were prepared for malaria microscopy.

Parasitological analysis and HIV test

The detection of malaria parasites wasperformed by preparing thick and thin films,staining with 10% Gienmsa, and examining with the microscope (Olympus Optical Co., Ltd, Japan)[19]. If the parasite was observed then the density was determined by counting the number of parasites against 200 leucocytes and multiplying the results by the actual white blood cell count of the patient [1].An applicator stick was used to take 1g of stool which was emulsified in 7mL of 10% formol water and 3mL of ether ina screw -cap tube using the fomol-ether concentration technique to detect for intestinal parasites, a process described by Cheersbrough[20].Blood samples were also used microscopically to detect for the HIV antibodies.Using a precision pipette 50 µL of specimen was collected from the subjects and applied to the absorbent pad on the Abbott Determine strip. For whole blood, only 1 drop of the chase buffer was added to the specimen pad and allowed to wait at room temperature for 15 minutes and results were read [90]. Determine rapid test results were read as follows:

Reactive: two lines of any intensity appeared on both the control and patient test areas.Non-reactive: one line appeared in the control area and no line in the patient area.Invalid: No line appeared in the control area. Invalid results shall not be reported.

Questionnaire

A pre-tested structured questionnaire was used to collect socio-demographic information; sex, age, residence, marital status, occupation while clinical data such as symptoms, HIV status, were collected from each patient. The knowledge, attitudes and preventive practices towards malaria and opportunistic intestinal parasites as well health seeking behaviours of participants were also recorded.

Data analysis

Data was logged intoMicrosoft excel, 2016 spreadsheet (Microsoft Corporation Inc, USA), and analysed with the Statistical Package for Social Sciencesversion 26.0(IBM-SPSS, Inc., Illinois, USA). Descriptive statistics were used to summarise the data. For Categorical data, frequency, counts, and percentages were used, and continuous variables were summarized with means, standard deviations (SD), and ranges. The association between categorical variables were tested using the Chi-square (χ^2) test.The Student T-test and ANOVA were also used to determined association in sample means. In the univariate logistic regression, a variable that showed association <0.2, significance level was considered in the multivariate analysis. A p-value of 0.05 or less was considered statistically significant unless stated otherwise.

Ethical consideration

The study was approved by the Institutional Review Board of the Faculty of health Sciences, University of Buea,Cameroon. Administrative clearance was obtained from the Regional Delegation of Public Health for the Northwest Region of Cameroon.All study procedures were conducted in strict adherence to good clinical and laboratory practices. All eligible participants were asked to provide their consent by signing an informed consent form before enrolment in this study.

Results

Study participant's demographic characteristics

A total of three hundred and sixty-seven (367) adult malaria patients were approached and introduced to the study. Three hundred and thirty-330 (89.9%) were successfully enrolled, provided the stool and blood samples, and completed the survey questionnaire. The mean age of the participants was 37.02(±15.235) years. There were 215 (65%) females and 115 (35%) males. A significant proportion of the participants were unskilled (79%), married (59.4%), and had attained a secondary level of education (39.1%). The highest significant number of

participantswith malaria were observed in the age group of<25 years. Summary of baseline demographic characteristics of the participants are shown (Table 1).

Table 1. Age specific distribution of malaria, and demographic factors (N=330)

		Age groups									
Variable	Subtype	< 25	26 - 35	36 - 45	46 - 55	56 - 65	≥ 66	Total n (%)	χ2	df	P. value
Gender	Male	40(12.1)	39(11.8)	16(4.8)	11(3.3)	4(1.2)	5(1.5)	115 (34.8)	12.510	5	**0.028**
	Female	63(19.1)	49(14.8)	43(13)	24(7.3)	27(8.2)	9(2.7)	215(65.2)			
	Total	103(31.)	88(26.7)	59(17.9	35(10.6)	31(9.4)	14(4.2)	330(100)			
Occupation	Skilled	23(6.7)	25(7.6)	13(4.3)	9(2.1)	4(1.2)	0	75(22)	8.139	5	0.149
	Unskilled	80(24.5)	61(18.7)	46(13.8)	28(8.6)	27(8.3)	14(4.3)	256(79)			
	Total	102(31.)	86(26.3)	59(18)	35(10.7)	31(9.5)	14(4.3)	333(100)			
Marital status	Married	22(6.7)	53(16.1)	55(16.7)	31(9.4)	26(7.9)	9(2.7)	196(59.4)	189.587	15	**0.001**
	Single	81(24.5)	34(10.3)	4(1.2)	3(0.9)	2(0.6)	1(0.3)	125(37.9)			
	Divorced	0	1(0.3)	0	1(0.3)	0	0	2(0.6)			
	Widowed	0	0	0	0	3(0.9)	4(1.2)	7(2.1)			
	Total	103(31.)	88(26.7)	59(17.9)	35(10.6)	31(9.4)	14(4.2)	330(100)			
Education	NFE	6(1.8)	8(2.4)	7(2.1)	8(2.8)	18(5.5)	13(3.9)	60(18.2)	122.297	15	**0.001**
	Primary	36(10.9)	36(10.9)	32(9.7)	13(3.9)	8(2.4)	0	125(37.9)			
	Secondary	59(17.9)	37(11.2)	16(4.8)	13(3.9)	3(0.9)	1(0.3)	129(39.1)			
	Tertiary	2(.0.6)	7(2.1)	4(1.2)	1(0.3)	2(0.6)	0	16(4.8)			
	Total	103(31.2)	88(26.7)	59(17.9)	35(10.6)	31(9.4)	14(4.2)	330(100)			

Clinical manifestation of malaria

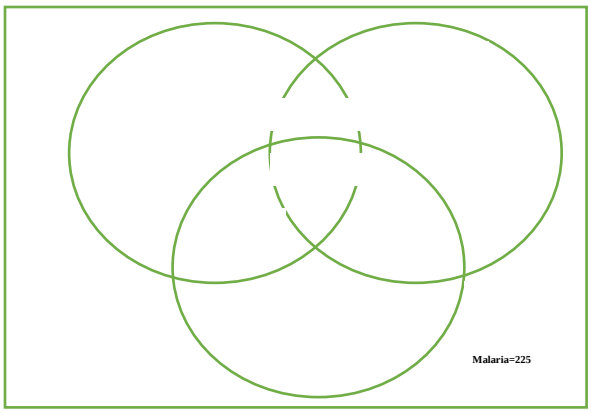
Fever was most reported in severe malaria 315/330 (95%), followed by those who reported abdominal pains 85/330 (26%), while diarrhoea was reported in 35/330 (11%) of the study participants. All three clinical symptoms appeared significantly associated with malariaP< 0.05 (Table 2). After controlling for confounders, abdominal pain negatively impacted on malaria intensity accounting for 4% of the dependent variable t (-2.531) (*P*-value=0.012).

Table 2: Relationship between clinical symptoms and malaria infection type (N=330)

Var.	Sub-variable	Malaria Type		(n)(%)	χ2	df	P-value
		Simple	Severe				
Fever	No	6	9	15(5)	5.9	1	0.027
	Yes	50	265	315(95)			
Abdominal pain	No	33	212	245(74)	8.3	1	0.004
	Yes	23	62	85(26)			
Diarrhea	No	46	249	295(89)	3.7	1	0.051
	Yes	10	25	35(11)			

Malaria co-infection with pathogenic intestinal parasites (PIP), and HIVvirus

Out of 330 participants, 64 were co-infected with coccidian intestinal parasites giving a prevalence of (19.4%). Malaria co-infection prevalence with other pathogenic gastro-intestinal parasites was 18/330 (5.5%), and co-infection with HIV was 27/330 (8.2%).The intestinalcoccidian parasites co-infections observed were; *Cryptosporidium hominis* which had the highest prevalence of 46/64 (13.9%) with malaria, followed by *Cycloisosporacayetanensis* oocysts with a prevalence of 13/64 (3.9%) with malaria and *Isospora belli* (also known as *Cystoisospora belli*), with a prevalence of 5/64 (1.5%) with malaria. The pathogenic intestinal parasites were helminths were [*Ascaris lumbricoides* (2, 0.6%), Hookworm (3, 0.9%), *Paragonimussp* (2, 0.6%), *Schistosoma mansoni* (1, 0.3%)]; other pathogenic protozoans found in the study were *Entamoeba histolytica* (4, 1.2%), *E. coli* (3, 0.9%), and *Giardia lamblia* (1, 0.3%)]; and fungi identified was Yeast cells (2, 0.6%). Overall, there were 109 malaria patient that were observed co-infected with either pathogenic intestinal parasites or HIV virus giving a co-infection prevalence of 109/330 (33%) (Figure 1).



N=330

Figure 1: Proportion of malaria co-infection with Opportunistic coccidian parasites, other intestinal pathogenic parasites, and HIV

Malaria co-infection withcoccidian and other gastro-intestinal parasites, and socio-demographic factors

Malaria co-infection prevalence with intestinal coccidian parasites was higher in females 45/64 (70%), than males19/64(30%). The highest prevalence of coccidian infection 14/64(22%), was found in the <25 years old age group (Table 3), and the lowest prevalence 6/64(6%) in the >66 years and older age group. The prevalence was more in the unskilled 50/64(78%) than the skilled participants. No significant association was observed between the age groups and the prevalence of the pathogenic coccidian parasites p-value>0.05.

Table 3: Age specific distribution of malaria co-infection with coccidian intestinal parasites (N=64)

	Coccidian intestinal parasites			Total (%)	χ^2	df	P-value
Age Group	*a*	*b*	*c*				
< 25	10	1	3	14(22)	7.2	10	0.705
26 - 35	6	1	3	10(16)			
36 - 45	11	0	2	13(20)			
46 - 55	8	0	3	11(17)			
56 - 65	9	2	1	12(19)			
≥ 66	2	1	1	4(6)			
Total	46	5	13	64			

a= Cryptosporidium hominis, b= Isospora belli c= Cycloisosporacayetanensis

However, *Cryptosporidium spp*, appeared to be widely distributed in all adult age groups showing the incidence in decreasing order as follows 36-45, <25, 56-65, 46-55, 26-35 and the ≥66 years and older. *Cyclospora spp* was also widely distributed across all the adult age groups, with similar incidence in the <25, 26-35, and 46-55 age groups, then follow by the 36-45 years, and the lowest incidence in the 55-66 and the ≥66 years and older both showing similar incidences. *Isosporaspp*, somehow showed a bi-polar distribution of occurrence in the age groups. Higher incidence was seen in the 55-65, then followed by lower but similar incidences in the <25, 26-35, and in the ≥66 years and older *Cycloisospora spp.* incidence was highest in the <25, and 25-35 years. *Isospora spp.* was completely observed absent in two age group categories; 36-45, and the 46-55(Figure 2).

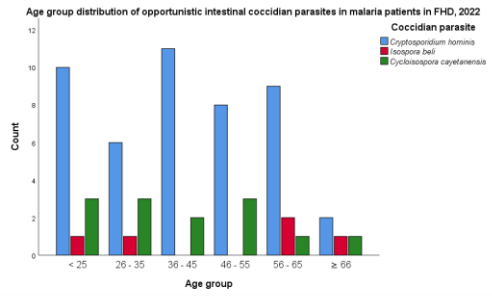


Figure 2: Age group incidence of coccidian infection and malaria

A similar malaria co-infection prevalence with other gastro-intestinal parasites was observed, declining co-infection with increase in age (Table 4). The highest parasite density was observed in <25 years or younger and progressively declined to the age group ≥66 years and above. There was no statistical significance between malaria co-infection prevalence with other pathogenic intestinal parasites and age.

Table 4: Age specific distribution of malaria co-infection with other gastro-intestinal parasites.

Age Group	Malaria	Other gastro-intestinal parasites(n)	%	χ^2	df	P-value
< 25	103	6	33	47.703	40	0.188
26 - 35	88	5	28			
36 - 45	59	2	11			
46 - 55	35	3	17			
56 - 65	31	1	6			
≥ 66	14	1	6			
Total	330	18				

The incidence of malaria co-infection with *Entamoeba histolytica* appeared to show a widespread distribution across the age groups from twenty (21) years to 55, but absent in the age group 56 years and above (Figure 3). Next is *E. coli* and *hookworm* which occurred in a similar incidences but hookworm infection is limited one age group of 25 years and below while *E. coli*turn to infect the 35-45 year age group. *Ascaris lumbricoides* was only observed in the older adults 56 years and older. *Giardia lamblia* was observed in 25-35 years age group as well as the fungal yeast cells. *Paragoniumsp* was in the 26-55 years age groups, *Shistosomasp* appeared in the 46-55 years age group. The highest malaria co-infection with pathogenic intestinal parasites was observed in the 25 years or lower age groups and the lowest in the 66 years and older. However no significant association was observed between the different age groups and incidence of gastro-intestinal parasites (Table 5).

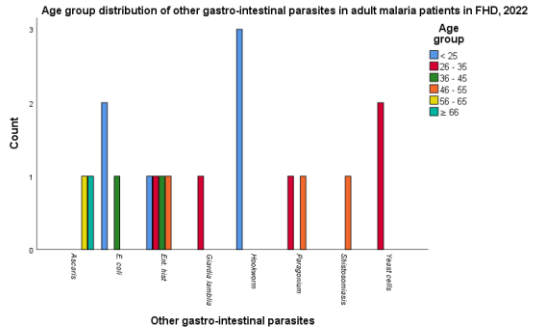


Figure 3: Age group incidence of pathogenic gastro-intestinal parasites and malaria co-infection.

The age group distribution of the malaria co-infections with HIV were negatively significantly associated as shown (Table 5).

Table 5: Age specific distribution of malaria co-infection with HIV virus (N=64)

Age Group	Malaria	HIV positive	%	χ^2	df	P-value
≥21< 25	103	6	22	16.286	10	0.092
26 - 35	88	7	26			
36 - 45	59	5	19			
46 - 55	35	7	26			
56 - 65	31	1	4			
≥ 66	14	1	4			
Total	330	27				

Malaria co-infection with HIV increased from <25 years age group up to 55 years and then sharply dropped and level off at 56 years and above. In decreasing order, the highest incidence of malaria co-infection with HIV were observed in the 26-35, and the 46-55 age groups with similar rates of incidence occurring. The next age group of <25 years or younger and 36-45, were observed with higher incidence rates. The lowest incidence rates were observed in the 56 years and older age groups (Figure 4). The highest malaria co-infection with HIV virus was observed in the 26-35 and 46-55 years age groups, and the lowest in the 56 years and older age groups.

Nosignificant association was observed between the different age groups and HIV viral infection.After controlling for confounding, HIV predicted for malaria. F (1) = 10.445, pvalue=0.002; which indicates 9.8% of the variance was explained by the model.

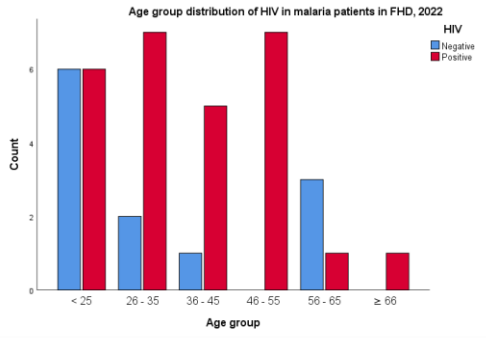
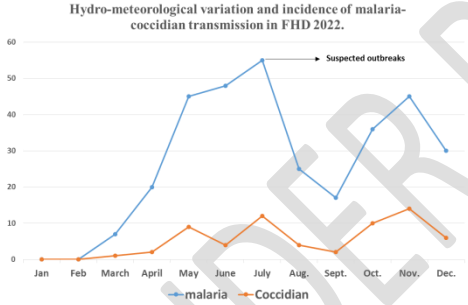


Figure 4: Age group incidence of malaria and HIV co-infection.

Hydro-meteorological factors and prevalence of malaria-coccidian co-infections

A time series analysis of hydro-meteorological factors and pathogenic coccidian concentration in malaria patients were used to identify periods of high contamination and infections from January to December 2022. Our study showed malaria and coccidian parasite co-infections were frequently detected during the rainy season's periods from March to September with peak period occurring between June, and July. July was observed most likely to have reported malaria and coccidian diseases outbreaks in the Health District (Figure 5).



Malaria and coccidian intestinal parasites travel related co-infections

Thirty-four 34/64(53%) co-infected patients found in the study reported travelling in/out of the Health District prior to consultation. A unidirectional movement as observed in their travel destinations as all of the patients reported travelling to the Southern parts of the country. In decreasing order, the Southwest 22/34(38), Littoral and the Centre regions of the country had similar score of 7/34(21%) each. Travelling to the other Health Districts within the Northwest region constituted 6/34 (18%). The Western Region was reported the least of travel destinations. None of the clinical symptoms was showed a significant relationship with coccidian parasitic infection. However, travel destinations were significantly associated with coccidian infections χ^2 (df) 29.47(10) *P* value=0.001 (Table 6).

Table 6: Summary of clinical signs, travel history and destinations in association with malaria-coccidian co-infection (N=64).

Variables	Sub-var	a	b	C	Total (n)	%	χ2	df	*P.* val
Fever	No	1	0	1	2	3	1.195	2	0.550
	Yes	45	5	12	62	97			
Abdominal pain	No	36	3	9	48	75	1.092	2	0.579
	Yes	10	2	4	16	25			
Diarrheal	No	39	3	9	51	80	2.813	2	0.245
	Yes	7	2	4	13	20			
Travel out of district in the last 2 weeks	No	20	3	7	30	47	1.736	2	0.420
	Yes	26	2	6	34	53			
Travel Destinations	SW	11	0	2	13	38	29.475	10	0.001
	LT	5	0	2	7	21			
	NW	3	1	2	6	18			
	CE	6	1	0	7	21			
	OU	1	0	0	1	3			

LT=Littoral, NW=Northwest, CE=Centre, OU=West a-*Cryptosporidium hominis*, b=***Isospora belli, c= Cycloisosporacayetanensis***

Factors predicting coccidian infections in malaria patients in FHD

A regression analysis was conducted determine for combination of factors responsible for coccidian infection in adult patients in the FHD. (Table 7). Results showed the source of drinking water, having a child in the house less than two years, treat water before drinking, method water treatment, household latrine have hand washing facility, hand washing using soap, keeping domestic animals, disinfecting animal farms for predicting coccidian infection. The results also showed, hand washing using soap, having children less than two years at home predicted coccidian infection in malaria patients. F (2, 64) = 7.375, p value=0.003; the adjusted R square was 0.149, which indicates 14.9% of the variance in the coccidian infections was explained by the model.

Table 7: Predictors of coccidian infection in adult malaria patients in FHD, 2022

Coefficients							
	Unstandardized Coefficients		Standardized Coefficients			95.0% Confidence Interval for B	
Model variables	B	Std. Error	Beta	t	Sig.	Lower Bound	Upper Bound
(Constant)	2.128	1.007		2.114	.039	.110	4.146
Child in household less than 2 years	**-.438**	**.212**	**-.259**	**-2.064**	**.044**	**-.863**	**-.013**
Treat water before drinking	.133	.528	.044	.252	.802	-.925	1.191
Water treatment method	**.278**	**.159**	**.259**	**1.747**	**.086**	**-.041**	**.598**
Household latrine has hand washing facility	.216	.222	.127	.971	.336	-.230	.661
Materials used for hand washing	**-.940**	**.329**	**-.437**	**-2.857**	**.006**	**-1.599**	**-.280**
Keeping domestic animals/pets	-.062	.240	-.032	-.258	.797	-.544	.420
Do you disinfect animal shed	.058	.193	.038	.299	.766	-.330	.446

However, results of the regression analysis showed, boiling water before drinking to remove diseases causing organisms, garbage waste being a breeding site for rodents and flies, human waste from filled latrine should be emptied in rivers or water bodies, OIPs can be found in flood waters, and defecating in water can lead to water borne diseases did not predict the attitude scores that were observed. F (6, 64=5.938, *p*-value=0.174.

Discussion

The current study aimed to determine the prevalence of malaria co-infections with pathogenic intestinal parasites (opportunistic coccidian parasites) and HIV virus and the associated risk factors in the FHD. For many years the Cameroon Government has been implementing a combined prevention and control strategies for malaria and pathogenic intestinal parasites through the national deworming programme. Although the prevalence of intestinal parasites in Cameroon has been on a decline from 33% in 2006 to 27.8% in 2012[21]. However, these strategies have not adequately address malaria co-infection with other pathogenic gastro-intestinal diseases cause by opportunistic pathogenic gastro-intestinal parasites in particular.

In our study, it was therefore important to generate updated data that public health officials can use in the implementation of evidence–based public health polices and more importantly use it to capacitate health personnel in emergency preparedness. The mean age of our study participants was 37.02 (±15.235) years, slightly higher than[6] and Njunda et al.[22] in a study in Yaoundéwho reported 35.5(±16.5), and 35.29(±12.26) respectively. Mean age of42.6±19.4) have also being reported in another study in Yaoundé [21]. In other countries a mean age of 37.7 was reported in Mozambique [23] in adult patients. In our study females had higher prevalence of malaria infection than males, in addition females had higher prevalence of malaria-pathogenic pathogenic gastro-intestinal parasite co-infection 45/64 (70%), than males 19/64 (30%). Affirming the findings of previous studies, where more females were infected with malaria than males carried out in Cameroon, Uganda, Nigeria and Ghana [6,24-27]. In our study antenatal visits by pregnant women and the ability of women to seek health accounted for the higher number of females participants explained the result obtained. However, it was contrary to studies in Cameroon and Equatorial Guineaand Australia [28-30] where males were more affected with opportunistic intestinal parasites than females. In Australia men with same sex (MSM) where coccidian infection was reportedly high 52% among MSM compared to

13% among non-MSM.In Cameroon and Equatorial Quinnea, socio-economic activities outdoors where men that exposed men to outdoor activities thereby exposing to mosquito bites at night[28].When the current study compared prevalence with age groups, the data showed that younger adults age 21-<25 years, had the highest prevalence of malaria in co-infections with coccidian parasites than the older adults and the lowest prevalence in the 66 years or older. This is contrary to the study in Yaoundé where finding showed women were most infected with PIPs and the age most affected with PIPs co-infected were people 32 years of age [21] and PIPs infestation was significantly distributed across the different age groups.Young people turn to remain outdoors more than older adults exposing themselves to mosquito bites. Young people also turn to have poor hygiene compared to older adult's thereby exposing them to the higher risk of acquiring intestinal parasitic infections. Moreover the highest prevalence observed suggest a delayed in acquiring natural immune response to both malaria and intestinal parasitic infections as a result of poor intervention in the early ages. Similar findings were reported Pokam et al.[31] in Mali[32] where higher malaria co- infections were reported in younger adult age groups than older adults. Furthermore when comparing malaria co-infections by area of residence, we showed that living in more rural setting was associated with higher risk of malaria-co-infection with PIPs, although this increase were not statistically significant. However the study revealed a significant associated between malaria co-infection with PIPs and travelling. Those who had reported having travel out of the study area before returning in the last 12 months were significantly at higher risk of malaria-PIPs co-infection than those who did not travel.The married participants 42/64 (66%), were also more co-infected with coccidian parasite than the singles.This is different from [6] who reported more coccidian infections in singles. Being married is often associated with children and the possibility of human-to-human transmission of coccidian parasites is high among lactating mothers or mothers with toddlers who are more in contact with the soil where they can easily pick up the oocyst.

Malaria-co-infection with coccidian intestinal parasites

A study suggestsintestinal parasites have modulatory effects on malaria infection pathophysiology [29]. The study investigated the prevalence of malaria in groups of individuals categorised with and without intestinal parasites. The analysis showed that the prevalence of opportunistic intestinal parasites (OIPs) in adult malaria patients was 64/330 (19.4%), even though the difference was not statistically significant with those who were not co-infected. This is similar to the finding in Buea, where no statistical significance was observed among adult patients infected with *Plasmodium* species and intestinal parasites and those who were not co-infected [6]. This prevalence is higher than the rate obtained in adult patient population in Bamenda[33], in which prevalence co-infection rate of (15.5%) was reported and in Buea a rate of 10.4% was reported[6]. In the centre Region of Cameroon, a rate of 22.1% prevalence was reported in Mfou, District[34]. In Melong and Denzo Littoral region of Cameroon a higher prevalence rate of 28.3% have been reported [35]. The prevalence in our study is higher than in Australia where a prevalence rate of 4% was also reported [23]. This results obtained are in line with our current knowledge where the prevalence of coccidian parasites is higher in less developed than developed settings.In this current study no significant association was observed between malaria infection and opportunistic coccidian infection (p value =0.233) or other pathogenic intestinal parasite (p value=0.767). This finding is concords with the study by Njunda et al.[22] who did not find any significant association between malaria and coccidian parasitic infections but contradict the study in Equatorial Guinea where the prevalence of malaria was significantly higher in patients that were co-infected with PIPs than in those who were not co-infected[29].In our study malaria infection was not significantly associated with age, gender, occupation, education (P value >0.05). Similar findings were revealed in earlier reports by [28-29], that found association between so-demographic factors and malaria infection. Fever, abdominal pains and diarrhoea were the most reported symptoms and were significantly associated with malaria intensity. Diarrhoea, fever and abdominal pains was significantly associated with malaria intensity and prevalence infection. This is similar to the study by Ngum et al.[37] who concluded that high parasitaemia contributes to malaria prevalence and severity.

When comparing malaria by the type of co-infections with coccidian intestinal parasites, we showed that in decreasing order malaria was most prevalent with *Crptosporidium hominis accounting for* (13.9%) of malaria infection, followed by*Cycloisosporacayetanensis* (3.9%), and least was with *Isospora belli* (1.5%). The evidence of association did not reach a level of statistical significance. Contrary to this finding, a significant association in prevalence of 8.9% protozoan in adults was reported in co-infection inYaoundéCameroon [21]. The large difference in sample size accounted for the different result obtained. In Nigeria a prevalence rates of *Crptosporidium hominis, and Cyclospora cayentensis, and Cystoisopora belli* were (41.1%), (28.9%) and 1(13.3%) respectively. In Mozambique a prevalence rate of 25%, 8.3% and 0% were reported for *Cryptosporidium Isospora and Cyclospora* oocysts respectively [38]. Prevalence rates also depends on a range of factors such as environmental risk factors, socio-demographic, and climate [37]. Our study showed prevalence rate of 0% in suburban settings, in contrast to a high prevalence rate of 22% observed in rural settings where hygiene and sanitation standards were low.

Malaria co-infection with other gastro-intestinal parasites.

When comparing malaria prevalence by the type of co-infections with other intestinal parasitic infections, data showed 5.5% prevalence of malaria patients were co-infected other gastro-intestinal parasites. The association did not reach a significant level. Rates of 3.8 %, 4.5 % and 26.4%, 34.2%, have been reported in different setting in Cameroon and in Africa [36, 39-40] and in the Middle East. The most frequently occurring helminth in our study was *Entamoeba histolytica* (1.2%). It was also the most frequent occurring in the Bamenda study where a higher a higher prevalence of 8% [33] was reported, and a similar study by Njunda et al. [1] but a much higher rate of 18.4% was reported. Elsewhere a lower prevalence rate of (0.5%) have been reported in Australia [41]. The prevalence of hookwormin our study was (0.9%). Higher rates of 1.4% reported in Buea and a significantly higher rate of 21.6% were reported in Mfou, a locality in central part of Cameroon [6, 34]. The prevalence of *Ascaris lumbricoides* a co-infectionwas0.6%. A higher prevalence rate of 2.2% was reported [35] in Melong. The prevalence of *G. lamblia* in our study was 0.3%. A higher rate of 31.5% was reported [42] in Tiko, and 15.2% reported in Munyenge[43], and 1% reported in Australian study [6]. The prevalence of *Shistostomamansoni*in our study was 0.3%. In other countries a prevalence of 1.3% have been reported in Ethiopia[44].Shistosomiais is associated with longer exposure in water and where flow rates are slow. Water bodies in our study area are very fast flowing rivers and will not favour the development of *Shistostomaspp*, thus it is suggested the patient may have one of the migrating patients and may have pick up the infection in another locality in Cameroon. Yeast cells, and *Paragoniumspp*, were also found in our study area, all with prevalence of <1%. No study have describe the co-infection between *Paragonimus*and *Plasmodium* species. However, a study has found yeast cells to rather have an inhibitory effect on malaria infection because of the toxin they produce which turns to a negative effect on the manifestation of *Plasmodium*species [45]. Malaria co-infections with multiples intestinal parasites are widespread and is likely an indication of transmission via the faecal oral routes, through contaminated foods, or water or other unhygienic practices [46]. It should be noted that occurrence and control of the coccidian parasites with malaria infections seems to have a correlation with COVID-19 which have proven effective control measures for a wide range of respiratory illnesses.

Malaria co-infection with HIV and AIDs virus.

The effect of malaria infection on HIV prevalence have also been established [47]. When comparing malaria prevalence by co-infections with HIV virus data showed that 8.2%prevalence of malaria were significantly co-infected with HIV virus, more of whom were women (85.2%).

The level of association reach reached a statistical significant level. Rates of malaria-HIV co-infections of 2.3% have been reported in Buea [48], and 14.1% was reported in Limbe[49]. At the Sub regional level rates of 18.5% and 47.7% have been reported in Nigeria [50]. These findings are similar to Sanyaolu et al.[47] who reported that increase in malaria and helminthic infection in HIV-infected women significantly lowers the CD4 counts level. Malaria infection often leads to breakdown of red blood cells (RBCs) resulting in anaemia [1, 6]. According to the Fundong Health District annual Report [not published] there are over 3000 individuals living with HIV and AIDS in the area. Moreover, pregnancy and routine compulsory HIV at antennal account for the higher number of females co-infected with HIV. Malaria co-infection with coccidian intestinal parasite was not significantly associated with any of the socio-demographic factors such as age, sex, education, age group, and residence. (P value>0.05). Similar finding, where revealed age, and education were not signification associated with coccidian co-infection with intestinal parasites and HIV have been reported[36]. Further investigations there was a significant negative relationship between malaria and HIV infections (P=0.001). Our results are in accordance with[6, 22], understandably so because lead to the destruction of the red blood cells thereby hindering the multiplication of the HIV virus that also use the red blood cells.

A time series analysis of hydro-meteorological factors and malaria and coccidian co-infection prevalence in adult malaria patients were used to identify periods of high contamination and infections from January to December 2022. Our study showed these pathogenic parasites were frequently detected during the rainy season's periods from March to September. However, the peaks events were not observed between Octobers to March (Dry season). The different hydro meteorological conditions had a noticeable impact on the annual distribution of coccidian intestinal parasites concentration in the environment as well as infection rate in adult patients. A similar study out by Sylvestre E. clearly shows hydro-meteorological factors influenced the monthly concentration of *Cryptosporidium* and *Giardia* in drinking water sources[52] in Canada where peak concentration was observed during snow melt period but a rapid decline in snow seems to lower the concentration of the parasites in drinking water sources. Our study area comprised of two major season-the rainy season from March to September. This period is characterised by heavy rainfalls, fast flow rate, high precipitations. Heavy rainfall and snowmelt can lead to short-term deterioration of

source water quality, and peak concentration of pathogens in drinking water have been recognized as the major cause of most water borne outbreaks[53], associated with drinking water, in the absence of/or inadequate treatment[54]. In our study low concentration were expected during the dry periods due to absence of rains where water tables are low and risk of contamination from latrine is low. Further no rainfall and no precipitation during the dry season implies no runoffs, no flooding, and risk of faeces from humans and animals being washed into water bodies are low thus lowering the concentration of the parasites in drinking water sources as well as the infectious dose in water bodies. This in turn lower the risk of human contamination.

In the current study, domestically acquired infections was 57.8% while 46.2% was among those reported recent travels in and out of the region prior to consultations. Those who had recent travels out of the region, coccidian infection was significantly associated to their travel destinations. These findings are similar to that reported in the US where 3.2% of coccidian infections were significant associated with recent travelling especially among immigrants coming from South Americas [55]. This also concords with Australian study where more than 10% of the study participants infected with coccidian parasites had reported oversea travels mainly within the Asia continent. Migration is frequently used as an important risk factor for protozoan infection monitoring in developed settings. Other studies have reported similar finding of coccidian infection among travellers [6, 55]. Coccidian diseases have associated with travellers especially from developed to developing countries or from urban setting to rural settings, where the hygiene and sanitary standards are low[56]. In this study, the regions reported by patients as their travel destinations have also been mapped out as high-risk areas in the country where water related outbreaks are frequently reported due to poor sanitation, flooding, water shortages[56,57]. This study revealed coccidian parasitic diseases were widespread in FHD with the highest prevalence of 22% observed. It revealed health areas such asBelo, Kikfuin, Anyajua, and Fuanantui had some of the highest prevalence of the coccidial diseases (16%, 19%, 19% and 22%) respectively. Crytosporidiosis and Cyclosporiasis *infections* were higher than Isosporiasis infection in the malaria patient population.

We studied 12 health areas and found coccidian parasite in 10/12 (83.3%) health areas. This concord to[34, 56], where co-infections with intestinal parasites were widespread. With *Cryptosporidium* species, thousands of oocysts are secreted in faeces and require just a little dose required for infectivity, compared to the *Isospora* and secreted in small quantities requiring a higher infective dosage and could possibly escape detection under the microscope. However, the size of *Cystoisopora* measures between (25-30) microns and a typical ellipsoidal shape[58]. Our study found 64.5% of all participation domesticated animals, a practice that facilitates the zoonotic transmission of the parasite from animals to humans [59]. The parasites have monoxenous development, and require a low infective dosage, and resistant to most disinfectants especially *Cryptosporidium*, reason why it can even be detected in water treated with chlorine[60-61]. Our findings showed *Cryptosporidium* and *cycloisospora*are more resistant and tend to have no preference for a particular month in its occurrence. This observation has been described by Lesley et al.[11] in which *Cryptosporidium* was observed not to have any trend in terms of seasonality. Outbreaks of *Cryptosporidium* are likely to occur anytime of the year, thus more likely to cause higher number of infections than *Isospora* and *Cyclospora*. *Cyclospora* was noticed completely absent in all water samples analysed as well as the low prevalence in stools samples obtained from malaria patients in the cross-sectional study compared to *Cryptosporidium* sp.

Conclusion

Malaria was significantly distributed in the different age groups and by gender, marital status and education. Malaria co-infection withintestinal coccidian and other pathogenic intestinal parasites in the area was influenced by migration immigration. Evaluation of malaria co-infection with intestinalcoccidian parasiteshave been neglected in clinical practice in FHD and nationwide in general. There is need to implement innovative combined intervention strategies in the control of malaria, PIPs and HIV virus.

Abbreviations

95%C.I; 95% Confidence Interval, AIDS; Acquired Immunodeficiency Syndrome, COVID-19; Coronavirus 2019, df; Degree of freedom, FHD; Fundong Health District, HIV; Human immunodeficiency virus, ITN; Insecticide Treated Nets, NTDs; NeglectedTropicalDiseases, OIP(s); Opportunistic intestinal parasite(s), RDT; Rapid diagnostic test, WHO; World Health Organisation, χ^2; Pearson's Chi-Square.

Data availability statement

All relevant data that supports the conclusion of this study are included in the article.

Ethic approval and consent to participate

The study protocol was approved by the Faculty of Health Sciences Institutional Review Board (FHS IRB). Administrative authorization was gotten from the Regional Delegation pf Public Health for the North West Region, the District Medical Officer(DMO) for Fundong Health District and Chiefs of centre for the various health facilities where data was collected.

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