Malaria, HIV, and Intestinal *Coccidian* Parasites Mixed-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 problems that maybe life threating. We studied opportunistic intestinal coccidian parasites (OIPs) and the human immunodeficiency virus (HIV) in adult patients with malaria in Fundong Health District, Northwest of Cameroon. The objectives was to determine prevalence, and distribution of the diseases in adult population.

Methods: 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: Three hundred and thirty (330) adult patients all infected with malaria took part in the study, 115(35%) males and 215 females (65%). The adult were of age 21 years and above. The mean age of participant was 37.02(±15.235) years. Malaria co-infection with coccidian parasites was observed in 19.4%. Malaria co-infection with other gastro-intestinal intestinal parasite capable causing pathological conditions in patients was 5.5%. The study showed the prevalence of malaria co-infections with HIV was 8.2%. The mixed infections observed were domestically acquired (57.8%) as well as travel related (46.2%). 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. Fever, and abdominal pain significantly associated with malaria status (simple or severe malaria) p-value=, P-value= respectively (5.915) 0.015, P-value= (8.3) 0.004, as well as the HIV status P-value= (11.798) 0.003. The odds of developing severe malaria from fever was 3.533(CI: 1.204-10.366) times higher compared to those who did not have fever. The odds of developing severe malaria from abdominal pain was 0.420(CI: 0.230-0.767) less. Results showed living with child<2 years, water treatment methods, material use in hand washing, predicted the probability of coccidian infection in the final logistic regression model accounting for 23.6% of the variance in the coccidian infections was explained by the model.

Conclusion: Prevalence of malaria, and opportunistic intestinal coccidian infections in adult patients was significantly influenced by migration, seasonal variation and the individual's immune status. Routine clinical practice have often ignore the investigation of possible mixed infections especially in persons with compromised immunity including HIV. Screening patients suspected of malaria should include investigation for opportunistic intestinal coccidian parasites especially for HIV and other vulnerable populations. An integrated prevention and control strategy need to be considered.

Keywords: Malaria-coccidian parasite co-infection; HIV; prevalence; migration; seasonal variation; screening; integrated control.

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; Neglected Tropical Diseases, OIP(s); Opportunistic intestinal parasite(s), RDT; Rapid diagnostic test, WHO; World Health Organisation, χ^2; Pearson's Chi-Square.

1. INTRODUCTION

Malaria and intestinal parasites are 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 malaria infection [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 controlled through Chemotherapy, 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 bear the greatest burden of parasitic diseases as compared to developed countries [6].This is due to the climatic conditions that favours the transmission of malaria and intestinal parasites, facilitating mixed infections in those living in the endemic zones.

Intestinal parasitic diseases occurring in co-infection with malaria include opportunistic intestinal coccidial diseases caused by intestinal coccidian parasites. Intestinal coccidian parasites include *Cryptosporidium species*, *Isospora species*, *Cyclospora species*, *Microsporidia species, Blastocystis species*, and *Toxoplasma specie*. *Plasmodium* species and intestinal coccidian parasites are both protozoans [7] in Pylum Apicomplexa. Susceptible persons are immuno-compromised (HIV/AIDs), those with underlying medical conditions, and children [1, 6-7].Studies on opportunistic intestinal parasites have been mostly focused on HIV, and on children [8-9]. Individual suffering from mixed infections of malaria and coccidian parasites can suffer from adverse health conditions ranging from mild to life threating [6].They are also at-risk of developing severe haematological problems and that sometimes could lead to death [1, 6, 10].

Although treatment for malaria using Artemisinin combination therapy (ACT), is largely available in Cameroon. Efforts even at the national level towards the prevention and control of these parasitic diseases have been slow. Intestinal opportunistic intestinal coccidian infections are among emerging diseases of public health importance in Cameroon. Thus the country health system is not yet prepared for the threat it may pose to the population. This makes the health system even more vulnerable, whereby in an event an outbreak they will be overwhelmed. Outbreaks have already been reported in USA and other countries like Italy. These have been associated with contaminated drinking water. Contaminated foods and sometimes animals have been implicated in their transmission [11-12].

Patients with mixed infections of malaria and opportunistic intestinal coccidian parasites are often not routinely evaluated in clinical practice. This allows the diseases to progress especially in immuno-comprimsed patients. The management of the diseases becomes complicated if they are not properly diagnosed. This study examines adult malaria patients for possible co-infections with opportunistic intestinal parasites in the Fundong Health District (FHD).The data will provide relevant information to policymakers in the area and in the country in the development of evidence based integrated interventions for prevention and control.

2. MATERIALS AND METHODS

2.1 Study Site

It is located between latitude 6° 4'and 6°23' to the North and longitude 10° and 10° 33' to the East. FHD is 80Km Northwest from the city of Bamenda. Its attitude ranges 800-2500m [13]. The area have different levels of urbanization. A majority of the population is settled in rural areas which make up about (80% compared to urban.The population is approximately estimated at 250,000 inhabitants. It area lies within the malaria high risk zones in Cameroon [4] with malaria prevalence estimated at 10% [14-16]. Malaria transmission is often exacerbated by poverty. This creates a favourable condition for malaria and intestinal parasitic diseases often refer to as diseases of the poor [17]. There is inadequate access to safe drinking water by the population, poor sanitation, and low socio-economic status. These conditions often expose the population to the risk of water borne infections, among them are opportunistic parasitic diseases in particular [6, 18].

2.2 Study Population

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

2.3 Specimen Collection and Processing

The signing of the consent form by participants was followed by health education on the purpose of the study and instructions on how to collect stool. Collect a teaspoon full sample into a labelled sterile stool container. About 4 mL 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.

2.4 Parasitological Analysis and HIV Test

Detection of malaria parasites was performed by preparing thick and thin blood films. They were stained with 10% Gienmsa, and examined under the microscope (Olympus Optical Co., Ltd, Japan)[19].The parasite 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 collect 1g of stool then, emulsified in 7 mL of 10% formol water and 3 mL of ether in a screw-cap tube using the fomol-ether concentration technique. The technique allows for the detection for intestinal coccidian parasite parasites and has been described by Cheersbrough [20].

Blood samples to detect 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.

2.4 Questionnaire

A pre-tested structured questionnaire was used to collect data on socio-demographic (sex, age, residence, marital status, and occupation). Data on clinical signs and symptoms (fever, diarrhoea, abdominal pains, and HIV were also obtained from participants. The knowledge attitudes and practices towards malaria and opportunistic intestinal parasites prevention were assessed. The health seeking behaviours of participants were also recorded.

2.5 Data Analysis

Data was logged into Microsoft excel, 2016 spreadsheet (Microsoft Corporation Inc, USA), and analysed with the Statistical Package for Social Sciences version 26.0 (IBM-SPSS, Inc., Illinois, USA). Descriptive statistics were used to summarise the data. Frequencies, counts, and percentages were used to summarize categorical variables. Continuous variables were summarized using means and standard deviations (SD).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 between 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 <0.05 was considered statistically significant unless stated otherwise.

3. RESULTS

3.1 Study Participant's Demographic Characteristics

Three hundred and sixty-seven (367) malaria patients were approached and introduced to the study, and three hundred and thirty-330(89.9%) participants were successfully enrolled. They all provided the stool and blood samples and completed the survey questionnaire. The mean age of the participants was 37.02(±15.235).The highest prevalence of malaria were observed in the age group of <25(31%) years.
This was followed by 26-35 years (27%) years. The third in prevalence was 36-45 years with 18%, then 46-55 years(11%), 56-65 years(9%) and >66 years(4%). There were 215 (65%) females and 115 (35%) males. There were (78%) unskilled participants as against 22% unskilled. Fifty nine (59 %) percent were married, thirty eight (38%) percent singles, one (1%) percent divorced and two (2%) percent widow. Eighteen (18%) had no formal education(NFE), while thirty eight (38%) percent completed primary school level, thirty nine (39%)completed secondary school and five(5%) had reached the tertiary level in their educational career.(Table 1).

3.2 Clinical Manifestation of Malaria and Symptoms

Fever was most reported in severe malaria 315/330 (95%). This was followed by abdominal pains with 85/330 (26%). Diarrhoea was the least 35/330 (11%) reported among study participants. The odds of developing malaria from patients with fever was 3.5(95% CI: 1.204-1.366) times higher compared to diarrhoea that was 0.462 (95%CI-0.208-1.026).Patients who reported abdominal pains were 0.420(95% CI: 0.230-0.767) less likely to develop malaria (Table 2).

3.2.1 Malaria co-infection with pathogenic intestinal parasites (PIP), and HIV virus

Out of 330 participants, sixty (64) four were co-infected with coccidian intestinal coccidian 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 intestinal coccidian parasites co-infections observed were; *Cryptosporidium hominis* which had the highest prevalence of 46/64 (13.9%) with malaria, followed by *Cyclospora cayetanensis* oocysts with a prevalence of 13/64 (3.9%) with malaria and *Isospora belli* with a prevalence of 5/64 (1.5%) with malaria (Table 3).

The other pathogenic intestinal parasites identified were helminths [*Ascaris lumbricoides* (2, 0.6%), Hookworm (*Ancyclostoma duodenale*) (3, 0.9%), *Paragonimus specie* (2, 0.6%), and *Schistosoma mansoni* (1, 0.3%)]. The protozoans identified in the study were *Entamoeba histolytica* (4, 1.2%), *E. coli* (3, 0.9%) and *Giardia lamblia* (1, 0.3%)]. A fungal yeast cell was also identified (2, 0.6%). A total of one hundred and nine (109) patients were observed co-infected with either pathogenic intestinal parasites or HIV virus, giving a co-infection prevalence of 109/330 (33%).

3.2.2 Malaria co-infection with coccidian and other gastro-intestinal parasites, and socio-demographic factors

Malaria co-infection prevalence with intestinal coccidian parasites was higher in females 45/64 (70%) compared to males who made up thirty 19/64(30%) percent. 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.

Table 1. Summary of socio-demographic characteristics of the participants (N=330)

						95% Confidence	
		N	%	Mean	SE	Lower Bound	Upper Bound
Age Group	>21<25	103	31	.79	.040	.71	.87
	26-35	88	27	.81	.042	.73	.90
	36-45	59	18	.83	.049	.73	.93
	46-55	38	11	.91	.048	.82	1.01
	56-65	35	9	.87	.061	.75	1.00
	>66	14	4	.86	.097	.65	1.07
Sex	Male	115	35	.85	.034	.78	.92
	Female	215	65	.82	.026	.77	.87
Occupation	Skilled	72	22	.83	.044	.75	.92

	Unskilled	255	78	.83	.024	.78	.87
Marital status	Married	196	59	.87	.024	.82	92
	Single	125	38	.76	.039	.69	.84
	Divorced	2	1	1.00	.000	1.00	1.00
	Widow	7	2	.86	.143	.51	1.21
Education	NFE	60	18	.83	.049	.74	.93
	Primary	125	38	.82	.034	.76	.89
	Secondary	129	39	.84	.033	.77	.90
	Tertiary	16	5	.79	.114	.54	1.03

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

		Malaria					
		Simple	Severe	Total (%)	Odds ratio (OR)	95% Confidence Interval	
Fever	No	6	9	15(5)	3.533	1.204	10.366
	Yes	50	265	315(95)			
Diarrhea	No	46	249	295(89)	.462	.208	1.026
	Yes	10	25	35(11)			
Abdominal pain	No	33	212	245(74)	.420	.230	.767
	Yes	23	62	85(26)			

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

	Age Group										95% CI for mean	
	< 25	26 - 35	36 - 45	46 - 55	56 - 65	≥ 66	Total	%	Mean	SE	Lower bound	Upper bound
Cryptosporidium hominis	10	6	11	8	9	2	46	13.9	3.13	.228	2.67	3.59
Isospora belli	1	1	0	0	2	1	5	1.5	3.80	.970	1.11	6.49
Cycloisospora cayetanensis	3	3	2	3	1	1	13	3.9	2.92	.445	1.95	3.89
Total (%)	14(22)	10(16)	13(20)	11(17)	12(19)	4(6)	64					

However, *Cryptosporidium specie*, 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 species* 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. *Isospora specie,* 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 *Cyclospora specie.* Incidence was highest in the <25, and 25-35 years. *Isospora specie* was absent in two age group categories; 36-45, and the 46-55(Figure 1). (*Something seems not right with these two adjacent paragraphs that I don't understand, you may adjust it please)*

A similar malaria co-infection prevalence with other gastro-intestinal parasites was observed. A declining co-infection was observed 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.

The incidence of malaria co-infection with *Entamoeba histolytica* appeared to show a widespread distribution across the age groups from between (21 to 55) years. It was however, absent in the age group 56 years and above (Fig. 3).*E. coli* and hookworm (*Ancyclostoma duodenale)* occurred similarly in different age groups. Hookworm (*Ancyclostoma duodenale),* infection was limited one age group of 25 years and below. *E. coli* tended to infect more the 35-45 year age group. *Ascaris lumbricoides* was only observed in older adults 56 years*. Giardia lamblia* was observed in 25-35 years age group as well as the fungal yeast cells. *Paragonium specie* was in the 26-55 years age groups, *Shistosoma specie* 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).

The odds of developing HIV infection from malaria was 1.146(95% CI: 0.039-13.886) times higher compared with those without malaria. For the cohort diagnosed with simple malaria they were 1.125(95%CI: 0.113-11.245) compared to the cohort diagnosed with severe malaria where the risk was 0.990 (95% CI: 0.810-1.211) (Table 5).

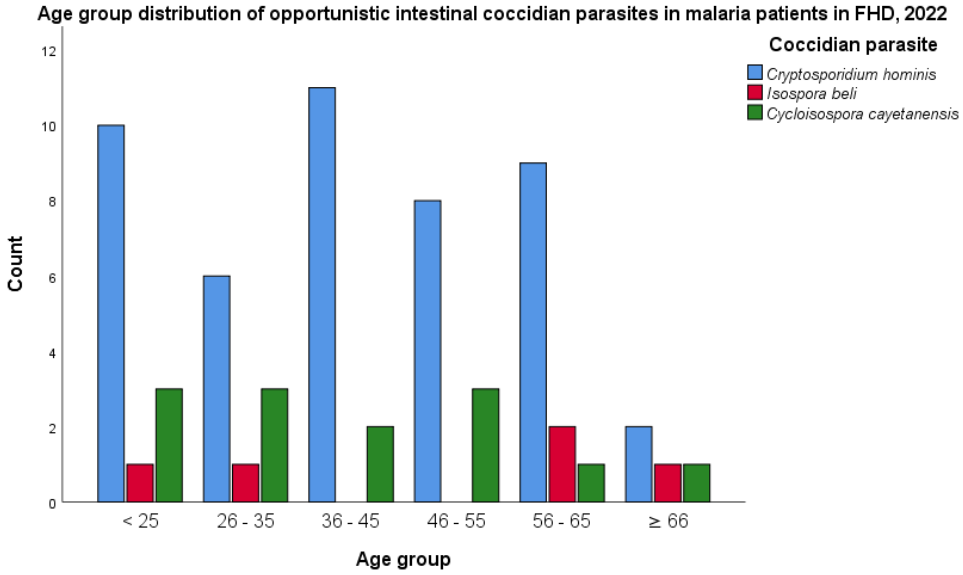


Fig. 1. Age group incidence of coccidian infection and malaria

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				

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

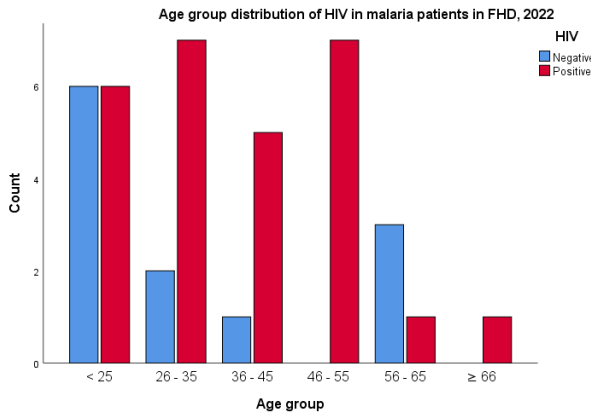
Age Group	95% CI for mean

	< 25	26 - 35	36 -45	46 - 55	56 -65	≥ 66	Total	Mean	SE	Lower bound	Upper bound
Malaria	103	88	59	35	31	14	330				
HIV + (%)	6 (22)	7(26)	5(19)	7(26)	1(4)	1(4)	27	.93	.051	.82	1.03

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. The highest and similar incidences of malaria co-infection with HIV were observed in the 26-35 and the 46-55 age groups respectively with both 26%. This was followed by the age group of <25 years with 22% and 36-45, with 19%.The lowest incidence were observed in the 56 years and older age groups (Fig. 2).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. No significant association was observed between the different age groups and HIV viral infection. After controlling for confounding, HIV predicted for malaria. F (1) = 10.445, p-value=0.002; which indicates 9.8% of the variance was explained by the model.

3.2.3 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, a 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 (Fig. 5).



3.2.4 Malaria and coccidian intestinal parasites travel related co-infections

Thirty-four 34/64(53%) co-infected patients found in the study reported travelling to other regions of the country prior to consultation. A unidirectional movement driven by economic factors was observed in their travel destinations as all of the patients reported travelling to the Southern parts of the country. The Southwest 22/34(38), Littoral and the Centre regions of the country had similar score of 7/34(21%) as travel destinations. Travelling to the other Health Districts within the region constituted 6/34 (18%).The Western Region was least reported travel destinations for participants. Our study observed that travel destination significantly associated with coccidian infections χ^2 (df) 29.47(10) *P* value=0.001.

3.2.5 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). We observed hand washing without using soap, and living with children less than two years at home predicted coccidian infection in malaria patients. F (2.405) = 9.859, P-value=0.032, which indicates 23.8% of the variance explained by the model. A decrease in coccidian co-infection score of .4 points with increase in the number of malaria patients living in homes without a child less than two years. Similarly in every good hand hand-washing practice using soap, the risk of coccidian co-infection score decreased by 0.9 points.

Fig. 2. Age group incidence of malaria and HIV co-infection

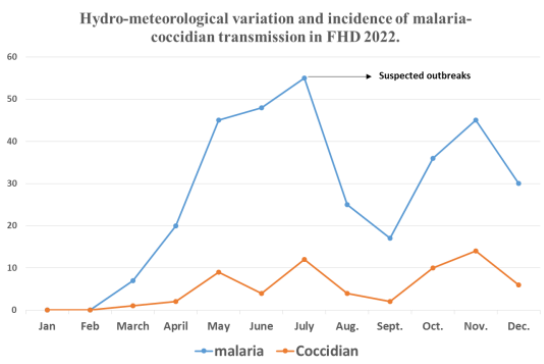


Fig. 3. Hydro-metrological variation and incidence of malaria coccidian transmission in FHD 2022

4. DISCUSSION

The current study aimed to determine the prevalence of malaria, opportunistic coccidian parasites and HIV mixed infections in the FHD. For years the Cameroon Government have implemented a combined prevention and control strategies for malaria and pathogenic intestinal parasites. The prevalence of intestinal parasites in Cameroon witnessed a decline from 33% in 2006 to 27.8% in 2012[21]. This strategy has not been able to adequately address malaria co-infection with other pathogenic gastro-intestinal including coccidian parasites.

In our study aimed to generate updated data for public health officials. They can use the data to implement evidence–based public health policies with significant positive impact on the health of the population. The information can also be used to build capacities of health personnel in emergency preparedness. The mean age of the study participants was 37.02 (±15.235) years. This was slightly higher than [6] and Njunda et al. [22] who reported the mean age 35.5(±16.5), and 35.29(±12.26) years respectively. Mean age of 42.6±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 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 Guinea and Australia [28-30] where males were found 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 exposed men to more outdoor activities thus exposure to mosquito bites [28].

The current study compared prevalence of malaria mixed infections with age groups and results showed that the younger adults age 21-<25 years had the highest prevalence of malaria and coccidian mixed infections compared to the rest of the older age groups. The lowest prevalence of malaria mixed infection was observed in the ≥66 year's age group. This is contrary to the study in Yaoundé, Cameroon where finding showed the age most affected with (IPs) co-infected were 32 years of age [21]. Intestinal parasites (IPs) infestation was significantly distributed across the different age groups. This findings was also similar to that reported by Pokam et al. [31] in Mali [32] where higher malaria co- infections were reported in younger adults than older adults.

Young people turn to remain outdoor compared to the older adults thus exposing themselves to increased mosquito bites. Young people also turn to practice poor hygiene when compared to older adults due to their strong ability to explore new environment. This exposes them to the higher risk of acquiring intestinal parasitic infections. This trend observed in prevalence of the co-infections also suggest a delayed in acquiring natural immune response to these infections that maybe as a result of poor intervention in the early ages.

By comparing malaria co-infections by area of residence, we showed that living in rural setting was associated with higher risk of malaria-co-infection with (IPs). The study revealed a significant associated between malaria co-infection with (IPs) and migration. Those who reported travelling out of the study area in the last two weeks prior to consultation were significantly at higher risk of malaria-(IPs) co-infection than those who did not travel.

The prevalence of mixed infection was higher in married participants 42/64 (66%), compared to the singles. This is contrary to the study by Kimbi et al [6] who reported a higher prevalence of malaria and intestinal parasite mixed in singles in Buea, Cameroon. Being married is associated with children at home. Children are among the vulnerable population most likely to be infected with intestinal coccidian infection due to their weak immune system. Living with children therefore increases the risk of human-to-human transmission and the risk is even higher among lactating mothers and mothers with toddlers. These category of children are more in contact with the soil where they are most likely to pick up the coccidian oocyst.

4.1 Malaria-Co-Infection with Coccidian Intestinal Parasites

A study suggests intestinal parasites and fungi infections 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. Fungi (yeast cells), have been found to have an inhibitory effect on malaria infection because of the toxin they produce which have a negative effect on the manifestation of Plasmodium species [33]. In addition, Toll-like receptors, such as receptors 2 and 4 (TLR2 and TLR4), play a considerable role in the host defense against microorganism [34]. TLR4 were associated with parasitemia and TLR2 receptors were related with malaria severity, while and none TLR was correlated with susceptibility [35]. Furthermore, Interleukin 10 (IL-10) is an immune-regulatory cytokine having anti-tumor effect [36]. It also promote anti-parasitic antibody production by B cells [37]. Anti-inflammatory cytokines such as transforming growth factor TGFβ1 and PDL-1[38-39] determine the magnitude of immune response following Malaria infection. [40].

Table 7. Predictors of coccidian infection in adult malaria patients in FHD, 2022(I thought this table was for the section 3.2.5)

Variable	Coefficients	95.0% CI	Pvalue	
Child in household less than 2 years	-.438	-.863	-.013	.044
Treat water before drinking	.133	-.925	1.191	.802
Water treatment method	.278	-.041	.598	.086
Household latrine has hand washing facility.	.216	-.230	.661	.336
Materials used for hand washing.	-.940	-1.599	-.280	.006
Keeping domestic animals/pets.	-.062	-.544	.420	.797
Disinfect animal shed.	.058	-.330	.446	.766

Our analysis showed that the prevalence of opportunistic intestinal parasites (OIPs) in adult malaria patients was 64/330 (19.4%). No statistically significant level was observed. This is similar to the finding in Cameroon where no statistical significance was observed among adult patients infected with *Plasmodium* species and intestinal parasites [6]. The prevalence obtained in our study is higher to that obtained in in a similar study in Bamenda [41] Cameroon, who reported (15.5%), and in Buea (10.4%) was reported [6]. In contrast, a higher rate of 22.1% was found in a study in the centre Region of Cameroon, in Mfou, District [42]. Similarly, a study in Melong and Denzo Littoral region of Cameroon obtained higher prevalence rate of 28.3% [43]. Elsewhere in Australia, a much lower prevalence rate of 4% was also reported [23].This results obtained are in line with our current knowledge where the prevalence of coccidian parasites tend to be higher in less developed than developed settings.

In this current study no significant association was observed between malaria infection and opportunistic coccidian infection (Pvalue =0.233), neither with other pathogenic intestinal parasite (Pvalue=0.767). This finding concords with the study by Njunda et al.[22] in Yaoundé, where no significant association between malaria and coccidian parasitic infections was observed. However, it contradict the study in Equatorial Guinea where the prevalence of malaria was significantly higher in patients that were co-infected with (IPs) [29]. No significant association was found between malaria and the socio-demographic such as age, gender, occupation, and level of education (P value= >0.05). Similar findings were revealed in earlier reports [28-29], which found no association between so-demographic factors and malaria. Fever was the most reported symptom and was found to be significantly associated with malaria intensity Pvalue=<0.05.This is similar to the study by Ngum et al. [44] who conclude 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 malaria co-infections with intestinal coccidian parasites was most prevalent with *Crptosporidium hominis accounting for* (13.9%), followed by *Cyclospora cayetanensis* (3.9%), and least was with *Isospora belli* with (1.5%), with no significant association observed. In contrary, a significant association in protozoan infection was

observed, in which a prevalence of 8.9% was reported [21].
The large difference in sample size accounted for the different result that were obtained. In Nigeria different prevalence rates of (41.1%), (28.9%), (13.3%) were reported for *Cryptosporidium hominis, and Cyclospora cayentensis,* and *Isopora belli,* respectively. In Mozambique a prevalence rates of 25%, 8.3%, and 0% were reported [45]. Prevalence rates also depends on a range of factors such as environmental risk factors, socio-demographic, and climate [46]. Our study showed prevalence rate of 0% in urban settings, in contrast to a high prevalence rate of 22% in rural settings. The urban setting have improved drainage system, better hygiene, and sanitary standards which contrast with rural settings where hygiene and sanitation standards were low.

4.2 Malaria Co-Infection with other Gastro-Intestinal Parasites

Our data showed 5.5% malaria patients co-infected other gastro-intestinal parasites. The association did not reach a significant level. Other studies have reported rates of 3.8 %, 4.5 % and 26.4%, 34.2% in different setting in Cameroon, Africa, and the Middle East [47-48]. The most frequently occurring helminth in our study was *Entamoeba histolytica* (1.2%). It was also reported most frequently occurring in the Bamenda study, where a higher prevalence of 8% [41] was reported. Similarly, Njunda et al. [1] reported a higher rate of 18.4%. Elsewhere, (0.5%) have been reported in Australia [48].
The prevalence of (*Ancyclostoma duodenale*) in our study was (0.9%). A higher rates of 1.4% have been reported in Buea, and a significantly higher rate of 21.6% were reported in Mfou, a locality in central part of Cameroon [6, 42]. *Ascaris lumbricoides* co-infection was 0.6%. lowere than 2.2% was reported[43] in Melong. The prevalence of *G. lamblia* in our study was 0.3%. A higher rate of 31.5% was reported [49] in Tiko, and 15.2% reported in Munyenge [50] both in Cameroon. In Australia 1% was reported [6].The prevalence of *Shistostoma mansoni* in our study was 0.3%. In other countries a prevalence of 1.3% have been reported in Ethiopia [51]. Shistosomiais infections have been associated with longer exposure in water, and where flow rates are slow. Water bodies in our study area are mostly fast flowing, due to sloppy nature of the landscapes.
A phenomenon does not favour the development of *Shistostoma species*. This suggest it might have been diagnosed in a patient with migrating behaviour pattern. The patient might have picked-up the infection in another locality. Yeast cells, and *Paragonium spp,* were also found in our study area, all with prevalence of <1%. No study have described the mixed between *Paragonimus* and *Plasmodium* species. However, a study found yeast cells to have an inhibitory effect on malaria infection because of the toxin with negative effect on the manifestation of *Plasmodium* species [33]. Malaria mixed with multiples intestinal parasites was widespread and overlapping in the area. This suggest a transmission via the faecal oral routes, contaminated foods, or contaminated water, and other unhygienic practices [52].It should be noted that the occurrence and control of the coccidian parasites with malaria infections seems to have a correlation with COVID-19 prevention and control measures, which also have a proven effective control measures for a wide range of respiratory illnesses.

4.3 Malaria Co-Infection with HIV and AIDs Virus

The effect of malaria infection and immune response have also been established [38-40, 53]. When comparing malaria co-infections with HIV, our data showed a 8.2% prevalence. More were women (85.2%) than men (14.8%), probably due to the routine HIV and malaria tests during antennal visits unlike men who only get to know their HIV status when ill. Lower rates of malaria-HIV co-infections have been obtained. In Buea, for example a rate 2.3% reported [54], and higher 14.1% in Limbe [55]. At the Sub regional level rates of 18.5% and 47.7% have been reported in Nigeria [56].Our study is similar to that of Sanyaolu et al. [53].
The study showed that an increased in malaria and helminthic co-infection in HIV infected women tends to significantly lower the CD4 counts levels. Malaria infection often leads to breakdown of red blood cells (RBCs) resulting in anaemia [1, 6]. Three thousand two hundreds (3200) individuals are living with HIV and AIDS, and receiving treatment (FHD annual report 2021 (unpublished) in the area. Malaria co-infection with coccidian intestinal parasite was not significantly associated with any of the socio-demographic factors (age, sex, education, age group, and area of residence) (P value=>0.05). Other studies have reported similar findings [57-58].

A time series analysis of hydro-meteorological factors and malaria and coccidian co-infection prevalence in adult malaria patients was carried

out. This was used to identify periods of high concentration of the parasites in the environment and between January and December 2022, and also to determine periods when outbreaks were most likely to occur. Our study results showed the parasites were frequently detected in patients during the rainy season (March-September). Low infections was observed between Octobers to March. Hydro-meteorological conditions had a noticeable impact on the annual distribution of coccidian intestinal parasites concentration in the environment. This also had a direct influence on different infection rates that were observed in patients.

Peak concentrations of the pathogens in drinking water were recognized as the major cause of most water borne outbreaks [60].The outbreaks often associated with drinking water in the absence of inadequate treatment [61]. A similar study by Sylvestre E. [59], showed hydro-meteorological factors influenced the monthly concentration of *Cryptosporidium* and *Giardia* in drinking water sources. Peak concentration of the parasite was observed during snow melt period. The study revealed a rapid decline in snow melt was associated with low concentration of the parasites in drinking water.

Heavy rainfall in the wet season and snow melt can lead to short-term deterioration of source water quality unlike in the dry season. The dry season is characterised by absence of runoffs, flood waters, and low risk of human and animal faeces being washed into water bodies. This tends to lower the risk of human contamination.

In the current study, domestically acquired infections was 57.8% while 46.2% was among those who reported recent travels in and out of the region two weeks prior to consultations. Intestinal coccidian infection was significantly associated to travel destinations. These findings are similar to that reported in the United States, where 3.2% of coccidian infections among were immigrants coming from South Americas [62]. In a similar study in Australian 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. There are other studies where similar finding [6, 62] have been reported. Coccidian diseases have been associated with travellers especially from developed to developing countries. Hygiene and sanitary standards are low [63] in developing countries as a result of non-enforcement of hygiene rules by authorities, thus allowing infectious diseases to thrive.

In this study the regions reported as frequent travel destinations are also high-risk areas in the country for water related outbreaks [63, 64]. Poor sanitation, flooding and water shortages have been frequently associated to these outbreaks.

This study revealed coccidian parasitic diseases were widespread in FHD with the highest prevalence of 22% observed in some health areas. It revealed the following health areas-Belo, Kikfuin, Anyajua, and Fuanantui had some of the highest prevalence of the coccidial diseases with rates of (16%,19%,19% and 22%) respectively. Crytosporidiosis and Cyclosporiasis *diseases* were higher than Isosporiasis in the population.We studied 12 health areas and found coccidian parasite in more than half of them 10 (83.3%). This concord with [11] who detected intestinal coccidian parasites in environmental water used by the population for wide range of purpose.

Their use includes drinking and other activities including recreational. *Cryptosporidium* species, secrets thousands of oocysts in faeces and requires a little dose required for infectivity. In contrary to *Isospora species* that secrets oocysts only in small quantities. This thus requires a high infective dosage. Because of this the parasite could possibly escape detection under the microscope. I*sopora belli* measures between 25-30 microns and has a typical ellipsoidal shape [64]. Our study observed that 64.5% of the participants domesticated animals. This practice is known to facilitate the zoonotic transmission of the parasite from animals to humans [65].

Coccidian parasites have a monoxenous development that requires a very low infective dosage. Intestinal coccidian parasites are also resistant to most disinfectants especially the *Cryptosporidium species*. This explains why they can be detected in drinking water even after treatment with chlorine [66-67]. Our findings showed *Cryptosporidium hominis* and *Isospora belli* showed no preference in its occurrence. Lesley et al. [11] described a similar observation, in which *Cryptosporidium* specie did not have any trend in terms of seasonality. Malaria mixed infection with *Cryptosporidium specie* is likely to occur anytime of the year. This in-turn is also more likely to cause higher number of infections than *Isospora species* and *Cyclospora species*. *Cyclospora species* was detected in any of the water samples analysed. There was also a low prevalence of the parasite in stools samples compared to *Cryptosporidium species.*

5. CONCLUSION

Malaria and intestinal coccidian mixed infections in Fundong Health District was significantly influenced by migration and seasonal changes. Malaria play a considerable role in host immune response in patients. Those patients that are immuno-compromized (HIV for example), have a higher risk of developing co-infections with opportunistic intestinal parasites.

Age, gender, marital status, and education significantly influenced the distribution of malaria and intestinal coccidian mixed infections in FHD. Clinical evaluation of malaria mixed infection with coccidian parasites have been neglected in clinical practice in general. There is need to implement innovative combined intervention strategies in the control of malaria, (IPs) and HIV virus.

CONSENT AND ETHICAL APPROVAL

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. All eligible participants were asked to provide their consent by signing an informed consent form before enrolment in this study.

ACKNOWLEDGEMENTS

We would like to thank all the staff of the 16 Health units for facilitating the field work throughout the study period. Our appreciation also goes to the administration of the Catholic General Hospital Njinikom for letting us use their Laboratory Services. Our appreciation goes to Mr. Teh Emmanuel and Mr. Young Augustine for assisting in the laboratory analyses. We wish to immensely acknowledge Mr. Frederick Nchang Cho of the Cameroon Baptist Convention Health Services, for inputs on data analysis and research methods. Special appreciation to all community members who accepted to participate in the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Njunda AL, Fon SG, Assob JCN, Nsagha DS, Kwenti TDB and Kwenti TE. Co-infection with malaria and intestinal parasites, and its association with anaemia in children in Cameroon. Infect Dis Poverty.2015;4:43.
Available:https://doi.org/10.1186/s40249-015-0078-5 PMID: 26445484
2. World Health Organization. World Malaria Report 2022. Geneva; WHO; 2022
3. WHO. Malaria fact sheet. Geneva: WHO; 2022
4. Antonio‐Nkondjio et al. Review of malaria situation in Cameroon. A technical viewpoint on challenges and prospects for diseases elimination. Parasites Vectors. 2019;12:501
5. Warren, “Cyclosporiasis: An Update,” in Current Infectious Disease Reports. 2009; 11:502–513
6. Mekachie Sandie S, Sumbele IUN, Tasah MM, Kimbi HK. Malaria and intestinal parasite co-infection and its association with anaemia among people living with HIV in Buea, Southwest Cameroon: A community-based retrospective cohort study. PLoS ONE. 2021;16(1): e0245743
7. Alemu G, Alelign D, Abossie A. Prevalence of opportunistic intestinal parasites and associated factors among HIV Patients while Receiving ART at Arba Minch Hospital in South Ethiopia: A Cross-sectional Study. Ethiop J Health Sci. 2018; 28(2):147
8. Polycarp N Chia et al. Assessment of *Cryptosporidium* infection in Benakuma health district of the Northwest region of Cameroon, Indian Journal of Medical Research and Pharmaceutical Science. 2018; 5(8).
Doi: 10:5281/zenodo.1404941
9. Kwenti TE, Nkume FA, Tanjeko AT, Kwenti TDB. The Effect of intestinal parasitic infection on the clinical outcome of malaria in coinfected Children in Cameroon. PLoS Negl Trop Dis. 2016;10(4): e0004673.
10. Nacher M, Singhasivanon P, Yimsamran S, Manibunyong W, Thanyavanich N, Wuthisen R. Intestinal helminth infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. J Parasitol. 2002;88(1):55–8
11. Lesley MB., Ahmad ST., Nur EY., Kasin A., Yvonee AL., Elexson N., Hashimatul EH. Detection of *Cryptosporidium* and *Cyclospora* oocysts from environmental water for drinking and recreational

activities in Sarawak, Malaysia. BioMed Research International;2017.
12. Frances Chelli et al. An outbreak of cryptosporidiosis associated with drinking water in north-eastern Italy. August, 2019: Microbiological and environmental investigations. Euro Surveill. 2022;27(35):
13. Kamga, F et al. Prevalence of onchocerciasis in the fundong health district, Cameroon after 6 years of continuous community-directed treatment with ivermectin. Pan. Afri. Med. Journl; 2011.
14. Massoda Tonye SG, Kouambeng C, Wounang R, Vounatsou P. Challenges of DHS and MIS to capture the entire pattern of malaria parasite risk and intervention effects in countries with different ecological zones: the case of Cameroon. Malar J. 2018;17:156.
15. MOH. Demographic and Health Survey. Summary Report; 2018.
16. National Institute of Statistic, Cameroon. Key results from the 2022 Cameroon Malaria Indicator Survey, CMIS; 2022.
17. Lifting Cameroons most vulnerable out of poverty building resilience and fostering local governance to address the root causes of fragility and conflict in northern regions of Cameroon; 2019.
18. Nsoh FA. Et al. Prevalence, characteristics and correlates of enteric pathogenic protozoa in drinking water sources in Molyko and Bomaka, Cameroon. BMC Microbiology. 2016; 16:268.
19. Berzosa et al. Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea Malar J; 2018
20. Cheesbrough M. District Laboratory practice in tropical countries. second ed. Cambridge: Cambridge University Press. 2006;178–235.
21. Vouking MZ, Enoka P, Tamo CV, Tadenfor CN. Prevalence of intestinal parasites among HIV patients at the Yaoundé Central Hospital, Cameroon. Pan Afr Med J. 2014;18.
22. Njunda AL, Njumkeng C, Nsagha SD, Assob JC, Kwenti TE. The prevalence of malaria in people living with HIV in Yaounde, Cameroon. BMC Public Health. 2016;16: 964.
23. Veronica Lambo., Marianne Lebbad., Salomao Maungate Johan Lindh., Occurrence of *Cryptosporidium spp*. And *Cystoisospora belli* among adult patients with diarrhoea in Maputo, Mozambique.. Heliyon. 2018;4.E00769.
24. Okiring J, Epstein A, Namuganga JF, Kamya, EV, Nabende I, Nassali, M.; Sserwanga, A.; Gonahasa, S, Muwema, M, Kiwuwa SM.; et al. Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. Malar. J. 2022; 21:22.
25. Quaresima V, Agbenyega T, Oppong B, Awunyo JADA.; Adu Adomah P, Enty E.; Donato F, Castelli F. Are Malaria Risk Factors Based on Gender? A Mixed-Methods Survey in an Urban Setting in Ghana. Trop. Med. Infect. Dis. 2021, 6, 161.
26. Jennifer O, Dogara M, Dogara M. Prevalence of malaria and risk factors among patients attending Dutse General Hospital, Jigawa State, Nigeria. Int. Res. J. Public Environ. Health. 2016;3:270–277.
27. Ibrahim AO, Bello IS, Shabi OM, Omonijo AO, Ayodapo A, Afolabi BA. Malaria infection and its association with socio-demographics, preventive measures, and co-morbid ailments among adult febrile patients in rural Southwestern Nigeria: A cross-sectional study. SAGE Open Med. 2022 22;10:20503121221117853.
28. Nyasa RB, Fotabe EL, Ndip RN. Trends in malaria prevalence and risk factors associated with the disease in Nkongho-Mbeng; a typical rural setting in the equatorial rainforest of the South West Region of Cameroon. PLoS ONE. 2021;16: e0251380.
29. Meñe GR, Mpina MG, Lopelo A, Nyakarungu EL, Bijeri JR, Elo AME, Ondo, F.A.; Garcia, G.A.; Phiri, W.P.; Ali, A.M.; Agobé, J.C.D.; Adegnika, A.A.; Abdulla, S.M. Effects of Age, Gender and Soil-Transmitted Helminth Infection on Prevalence of *Plasmodium* Infection among Population Living in Bata District, Equatorial Guinea. Trop. Med. Infect. Dis. 2023;8:149. Available:https://doi.org/10.3390/tropicalmed8030149
30. Ramdzan AR, Ismail A, Mohd Zanib ZS. Prevalence of malaria and its risk factors in Sabah, Malaysia. Int. J. Infect. Dis. 2020;91:68–72
31. ACN Djieyep, BT Pokam., DL David. Pokam-Prevalence of intestinal coccidian burden in HIV/AIDS patients on antiretroviral therapy in HIV centres in Mubi, Nigeria. African journal of clinical

and experimental microbiology. 2014;15(3) 165-172.
32. Coulibaly D, Guindo B, Niangaly, A.; Maiga, F.; Konate, S.; Kodio, A.; Diallo, A.; Antar ATM, Kone AK, Traore K, et al. A Decline and Age Shift in Malaria Incidence in Rural Mali following Implementation of Seasonal Malaria Chemoprevention and Indoor Residual Spraying. Am. J. Trop. Med. Hyg. 2021, 104, 1342–1347. [CrossRef] [PubMed
33. Valzan M., Cicarini V, Capelli A et al. A yeast strain associated to Anopheles mosquitoes produces a toxin able to kill malaria parasites.. Malar J. 2016;15(21).
34. Abdel Hammed MR, Elgendy SG, El-Mokhtar MA, Sayed D, Mansour SM, Darwish AM. T-lymphocytes Expression of Toll-like Receptors 2 and 4 in Acute Myeloid Leukemia Patients with Invasive Fungal Infections. Mediterr J Hematol Infect Dis. 2022 Mar 1;14(1):e2022022. DOI:10.4084/MJHID.2022.022. PMID: 35444773; PMCID: PMC8992612.
35. Ramirez Ramirez AD, de Jesus MCS, Rossit J, Reis NF, Santos-Filho MC, Sudré AP, de Oliveira-Ferreira J, Baptista ARS, Storti-Melo LM, Machado RLD. Association of toll-like receptors in malaria susceptibility and immunopathogenesis: A meta-analysis. Heliyon. 2022 22;8(4):e09318. DOI:10.1016/j.heliyon.2022.e09318. PMID: 35520620; PMCID: PMC9065626.
36. Mohammed D, Khallaf S, El-Naggar M. et al. Interleukin-10: A Potential Prognostic Marker in Patients with Newly Diagnosed Multiple Myeloma. Research in Oncology. 2021;17(1): 38-41. DOI: 10.21608/resoncol.2021.51503.1127
37. S Biswas · 2022 01 Autoimmune and Autoinflammatory Disorders. Front. Immunol.2022;13. Available:https://doi.org/10.3389/fimmu.2022.970906
38. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. *Int J Mol Sci* (2019) 20(23):6008. doi: 10.3390/ijms20236008.
39. Lokau J, Garbers C. Biological functions and therapeutic opportunities of soluble cytokine receptors. *Cytokine Growth Factor Rev* (2020) 55:94–108. doi: 10.1016/j.cytogfr.2020.04.003
40. Mamoru Niikura, Shin-Ichi Inoue, Fumie Kobayashi, "Role of Interleukin-10 in Malaria: Focusing on Coinfection with Lethal and Nonlethal Murine Malaria Parasites", BioMed Research International, vol. 2011, Article ID 383962, 8 pages, 2011. https://doi.org/10.1155/2011/383962
41. Bessong et al. Burden of intestinal parasites among HIV/AIDs patients attending the Bamenda Regional Hospital in Cameroon. African Journal of clinical and experimental microbiology.2015 (16)3.
42. Nkassomo. Zeukeng F, Tchinda VHM, Bigoga JD, Seumen CHT, Ndzi ES, Abonweh G, et al. (2014) Co-infections of Malaria and Geohelminthiasis in Two Rural Communities of Nkassomo and Vian in the Mfou Health District, Cameroon. PLoS Negl Trop Dis 8(10): e3236. 163.
43. Yamssi Cedric, Npoumedem Anangmo., Christelle Nadia, Vincent Khna Payne, Sabi Bethrand, Ngangnang, Romeo. Gastro-intestinal nematodes among residents of in Melong, Moungo division, littoral region, Cameroon.2021. BioMed. Research International.
44. Ngum, N.H., Fakeh, N.B., Lem, A.E. et al. Prevalence of malaria and associated clinical manifestations and myeloperoxidase amongst populations living in different altitudes of Mezam division, North West Region, Cameroon. Malar J. 2023;22(20) Available:https://doi.org/10.1186/s12936-022-04438-6 patients aged 36-60 years recorded significant distribution of malaria.
45. Neyder Contreras-Puentes, Diana Duarte-Amador, Dilia Aparicio-Marenco et al. Intestinal Coccidian: An overview epidemiology worldwide and Colombia. University Corporation Rafael Nunez, Faculty of Sciences of Health, Medicine, Colombia; 2019
46. Ngum Helen Ntonifor, Abongwe Sidney, Warra Tamufor, Lem Edith Abongwa. Prevalence of intestinal and associated risk factor in HIV positive and negative patients in Northwest Region, Cameroon.. Sci Report. 2022;12:16747.
47. Ketchazoue GG Igore, Vincent Khan Payne, Noumedem, AC Nadia, Yamssi Cedric. Risk factors associated with Prevalence and intensity of gastro-intestinal within households in Tonga Sub Division, West Region, Cameroon. J infect Dis. Epidemiol. 2020;6(3).
48. Stephanie Flectcher, Graziella Caprarelli, Juan Merif, David Andresen. Epidemiology and Geographical Distribution of enteric Protozoan infections, Australia. Journal of Public Health Research. 2014;3:298.
49. Adeline Enjema, Green Judith, Kuoh Anchang Kimbi, Godlove Bunda Wepnji,

Vicky Donyle Ndassi and Helen Kuokuo Kimbi. Distribution and factors associated with Uriniginal Schistosomiasis in the Tiko Health District. A semi-Urban Setting. South West Region, Cameroon. Green et al. Infectious Dieses of Poverty. 2021; 10:49.

50. Judith K. Anchang-Kimbi, Dillys Mansoh Elad, Gemain Taiwe Sotoing, Eric Akum Achidi, Coinfection with Schistosoma haematobium and *Plasmodium falciparum* and Anaemia Severity among Pregnant Women in Munyenge, Mount Cameroon Area: A Cross-Sectional Study", Journal of Parasitology Research, Article ID 6173465, 2017;12.
51. Slomon M Abay, Mulugeta Tilahun, Nigun Fikrie. Abiy Habtewold. Plasmodium falciparium coinfection and Shistosoma mansoni coinfection and their side benefit of arthemeter lumifantrine in malaria patients. Journal of Infection in Developing Countries.2013;7(6) 468-74
52. Paniaqua G, Monroy E, Garcia-Gonzalez O, et al. Two or more enteropathogens are associated with diarhoea in Mexican children. Ann Clin Antmicrob 2007,6:17
53. Sanyaolu AO, Fagbenro, -Beyioku, Oyibo WA, Badaru OS, Onyeabor OS, Nnaemeka CI. Malaria and HIV co-infection and their effect on haemoglobin levels from three healthcare institutions in Lagos, Southwest. Nigeria. Afr Health Sci. 2013;13(2):295-300.
54. Fru Georgia et al. Malaria and human-immunodeficiency virus co-infection in febrile patients attending the Regional Hospital of Buea, Southwest Region, Cameroon.. International Journal of advance medical and Health Research. 2019;6(2):46-51.
55. Sandie SM, Sumbele IUN, Tasah MM. et al. Malaria parasite prevalence and Haematological parameters in HIV seropositive patients attending the regional hospital Limbe, Cameroon: A hospital-based cross-sectional study. BMC Infect Dis2019;19(988)..
Available:https://doi.org/10.1186/s12879-019-4629-4
56. Olusola Ojurongbe et al. Prevalence of *plasmodium falciparum* parasitaemia and its correlation with haematological parameters among HIV-Positive individuals in Nigeria. Journal of Tropical medicines; 2014.
57. Izadi S, Ghayour-Najafabadi Z, Yavari M, Mohaghegh M, Wannigama D L, et al. Intestinal Parasites Associated with Opportunistic Coccidial Infections Among Immunocompromised Individuals in Central Iran: A Cross Sectional Study. Arch Clin Infect Dis. 2019;14(2):e79701
58. Van den Berk GE, Frissen PH, Regez RM, Rietra PJ. Evaluation of the rapid immunoassay determine HIV 1/2 for detection of antibodies to human immunodeficiency virus types 1 and 2. J Clin Microbiol. 2003;41(8):3868-9.
59. Sylvestre É. Systematic assessment of microbial risks associated with hydrometeorological events for drinking water safety management [Ph.D. thesis, Polytechnique Montréal]. Poly Publie; 2020.
Available:https://publications.polymtl.ca/5485/
60. Hrudey S, Hrudey E. Common themes contributing to recent drinking water disease outbreaks in affluent nations. Water Supply. 2019;19:1767-1777.
61. Hrudey SE, Hrudey EJ. Safe drinking water. Lessons from recent outbreaks in affluent nations. International Water Association Publishing, London, United Kingdom; 2004.
62. Sorvillo FJ, Lieb LE, Seidel J, Kerndt P, Turner J, Ash LR. Epidemiology of isosporiasis among persons with acquired immunodeficiency syndrome in Los Angeles County. Am J Trop Med Hyg. 1995 Dec;53(6):656-9.
63. CDC. Domestically acquired cases of cyclosporiasis – United States; 2018. Available:https://www.who.int/emergencies/disease-outbreak-news/item/2022 DON374.
64. Jungiang Li., Zhoaoui CUI., Longhxian Zhang: Advances in Cyclosporiasis Diagnosis and Therapeutic Intervention Clinical Microbiology Volume 10. 2020.
65. Ntazana N. Sinyangwe, Joyce Siwila, John B. Muma Chola, Charles Michelo. Factors associated with *Cryptosporidium* Infection among adult HIV positive Population in contact with livestock in Namwal District, Zambia. Frontier Public Health. 2020;8. Available:https://doi.org/10.3389/fpubh.2020.00074
66. Sun T Ilardi CF, Asnis D. et al., Light and electron microscopic identification of *cyclospora* species in the small intestine: Evidence of the presence of asexual life cycle in human host,". American Journal of Clinical Pathology, vol. 1996;105(2):216–220.

67. Tzipori S, Ward H. Cryptosporidiosis: Biology, pathogenesis and disease," 2002. Microbes and Infection. 2002;4:1047–1058