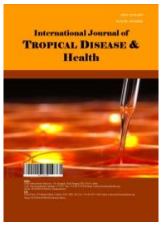

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Prevalence Rate of Intestinal Parasites/Malaria Co-Infections and their Associated Risk Factors in Melong and Denzo, Littoral Region- Cameroon)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EEJE and YW conceived and designed the study. Author MS collected data and took part in data analysis. Author GT analysed the data. All authors wrote the manuscript. Authors EEJE and YW supervised, reviewed and provided inputs to the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To determine the prevalence rate of intestinal parasites/malaria co-infections and their associated risk factors in Melong and Denzo, Littoral region- Cameroon

Materials and Methods: Study period was from November 2019-March 2020. Blood samples were collected after informed consent by finger pricking. Stool samples were examined using normal saline and the Kato-Katz technique for the presence and intensity of IPs. Thick blood films were prepared, Giemsa-stained and examined under x100 for the presence of parasites and estimate GMPD. A structured questionnaire was filled out to obtain information on different factors which might predispose participants to become infected. Data was analysed using SPSS version 23 at $P<0.05$.

Results: The overall prevalence of IP was 28.3% (113/400). *Entamoeba histolytica* was the most prevalent IP 22.0% (88/400) and it was significantly more in Melong (27.7%, 76/274) than in Denzo (9.5%, 12/126), ($P=0.001$). The overall prevalence of malaria was 66.5% (266/400). The prevalence

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of malaria was higher in Denzo (79.3%, 100/126) than in Melong (60.5, 166/274), P=0.001. Children ≤5 years recorded the highest (75.4%, 83/110) prevalence of malaria, and the difference between age groups was significant, p=0.016. The overall prevalence of co-infections was 16.3% (65/400). Low access to bed nets, presence of bushes and water bodies, poor drinking water source and lack of personal and community hygiene are the associated risk factors leading to co-infection of malaria and intestinal parasites.

Conclusion: Intestinal parasites/malaria co-infections are still a cause of morbidity and mortality in Cameroon; interventions targeting groups at risk will help reduce the burden of these diseases in Melong and Denzo localities.

Keywords: Intestinal parasites; malaria; co-infections; melong, denzo; littoral region; Cameroon.

1. INTRODUCTION

Intestinal parasites (IPs) infect the gastrointestinal tract of humans and animals. These infections comprise both helminthes and protozoans which form the most common infections worldwide [1]. It has also been estimated that about 3.5 billion people in the world are infected with intestinal parasites of which 450 million are ill [2,3]. According to the London School of Hygiene and Tropical Medicine [4], the most frequent helminths species are *Ascaris lumbricoides, Enterobius vermicularis, Hymenolepis spp, Trichuris trichiura, Strongyloides stercolaris, Schistosoma mansoni, Taenia* spp and hookworms. While most frequent protozoans are *Entamoeba histolytica and Giardia intestinalis.* These parasites modify gastrointestinal conditions and produce a variety of symptoms in infected persons such as, inflammation of the small and large intestine, diarrhoea/dysentery, abdominal pain and nausea/vomiting [5]. Most intestinal parasites cause infection that lead to acute and chronic diarrhoea in healthy individuals and may lead to life threatening illness in patients with immunosuppressive disease like HIV/AIDS.

Malaria is a disease transmitted by infected female *Anopheles* mosquitoes during their blood meal. The disease is endemic in all the 10 Regions of Cameroon with a percentage prevalence of about 20% [6]. Five species of *Plasmodium* cause malaria; *Plasmodium malariae, P. vivax, P. ovale, P. knowlesi* and *P. falciparium* with the latter being the most virulent, accounting for the majority of malaria deaths [7].

Co-infection of malaria and intestinal parasites is common in individuals in developing countries [8] and severe infections can lead to blood loss, tissue damage, spontaneous abortion and death. However, variation in the prevalence of intestinal parasites and malaria can be related to some factors such as, climate, geographical localization, hygiene as well as a variety of cultural, economic and social variables [9].

The inhabitants of Melong and Denzo localities are faced with the challenge of the factors that favour the transmission of these parasites which are poor sanitation, poor drainage and poor quality of water. In addition to these factors, Melong is located at a cross-road from Bamenda to other major towns like Nkongssamba, Douala, Buea and Limbe and it is an area where travelers usually stop to take a break consuming different types of foods and water which might promote propagation of intestinal parasites.

The findings from this study will provide a better understanding of intestinal parasites and malaria co-infection situation and provide baseline data on these parasites as this will add knowledge about malaria and intestinal parasites transmission and influence policy to improve community health. This study was designed to assess the prevalence of intestinal parasites and malaria co-infection as well as to determine the different factors which predispose people to become infected with the infections in Melong and Denzo, Littoral Region of Cameroon.

2. MATERIALS AND METHODS

2.1 Description of Study Site

Melong is a semi-urban area found in the Littoral Region of Cameroon, located at latitude 5^o 7’18N; longitude 9^o 57’41”E with an altitude of 760 m a.s.l. It has an average temperature of 19^oC and the climate is marked by two seasons, the rainy and dry seasons. It has an estimated population of 102,000 inhabitants [10] and the area is characterized by poor hygienic conditions due to presence of bushes and poor drainage systems. Inhabitants are engaged in various socio-economic activities.

Denzo, also known as New Melong is a rural area, one of the 40 villages found in Melong sub-division. It is divided into two ethnic groups headed by a chief. It is made up of 5,099 inhabitants [10].

Farming and trading are the main economic activities of the people in these localities.

2.2 Study Design and Study Population

This study was a cross-sectional examination in which the prevalence of intestinal parasites and malaria were determined. The study population was made of patients of all age groups, both males and females, that presented stool and blood samples for analysis in the Melong District Hospital and Denzo Integrated Health Centre.

2.3 Inclusion Criteria

Only patients that brought stool samples for analysis and blood for malaria parasite examination in Melong District Hospital and in Denzo Integrated Health Centre and had signed the informed consent form participated in the study.

2.4 Sample Size Estimation

The sample size of this study was calculated based on a similar prevalence study by Williams et al. [11] and the Cochran formula [12] were used. The formulation is:.

$$n=Z^2pq/ e^2$$

Where

- Z= standard number deviate (1.96 for a 95% confidence value).
- n = desired sample size
- P= prevalence of intestinal protozoan 42.9% [11].
- q= 1-p (proportion in the population that does not have the characteristics being measured).
- e = desired level of precision 0.05.
- n= $(1.96)^2(0.429)(0.571)/(0.05)^2$
- n= 376 participants.

It was calculated that a minimum sample size of 376 participants was required. A total of 400 participants were sampled to minimize bias.

2.5 Stool Collection and Examination

Patients were given clean, dry and air tight bottles labeled with their initials. They were given instructions on how to collect the required stool sample. A macroscopic examination was done to determine the presence of adult worms in the stool, consistency, and the colour. Direct microscopy examination was done using normal saline. The slides were observed under x10 objective and x40 objective lens of the microscope to detect trophozoite and cyst forms especially the motile forms. The Kato-katz concentration technique was used following the Tadesse et al. [13] and WHO [14] methods to determine the presence and intensity of helminth eggs.

2.6 Blood Collection, Preparation and Staining of Blood Smears

Prior to blood sample collection demographic information such as the age, sex, area of residence were recorded. Using sterile disposable lancets, finger pricks were performed. Thick and thin blood films were prepared using the method described by Cheesbrough [15]. The code number of each individual was written on the slide and the blood films were allowed to air dry protected from dust and flies. In the laboratory, the thin films were fixed with 100% methanol for one minute and both thick and thin blood films were stained with 5% Giemsa stain solution for 30 minutes [15].

2.7 Detection and Estimation of Parasitaemia of *Plasmodium* Species

The slides were read under x100 (oil immersion) objective of the microscope for the detection of malaria parasites by an experienced microscopist. A second experienced microscopist, blinded to the first reading, read all thick smears and any discrepancies (positive vs. negative; results that did not match each other; >25% difference in parasite density) were resolved by a third microscopist. Parasite densities were determined from thick blood smears by counting the number of asexual parasites or sexual parasites per 200 WBCs and converted to number of parasites/µl blood assuming a standard WBC count of 8,000/µl. A smear was considered negative if no parasites were seen after a review of 100 high-powered fields.

2.8 Administration of Questionnaires

A structured questionnaire was prepared in English and French. Two hundred and sixty nine (269) questionnaires were administered in the two localities which helped to record socio-demographic characteristics and information concerning risk factors associated with the spread of malaria and intestinal parasitic infections in the study areas. The questionnaires were filled by patients in the two medical facilities and others by individuals selected at random in the quarters of the two localities.

2.9 Data Analysis

Data collected were recorded in Microsoft excel. The data was analysed using the Statistical package for social sciences (SPSS) version 23. Proportions were calculated to obtain the prevalence rates of infection. Chi Square contingency test was used to test for significant differences in proportions. The Mann-Whitney test was used to check for significant differences in the geometric mean parasite density (GMPD) between localities and also between males and females. The Kruskal-Wallis test was used to test for significant differences in GMPD between age groups. Binary logistic Regression was used to assess the association between intestinal parasites and malaria-intestinal parasites co-infection and various factors. All the tests were performed at the 5% significance level. The data were presented in tables and figures.

3. RESULTS

A total of 400 persons were enrolled into the study (Table 1). In Melong, 274(68.8%) were sampled while 126(31.5%) were sampled in Denzo. More females (248, 62%) were enrolled into the study than men (152, 38%). The highest number of participants 110(27.5%) belonged to the ≤5 years age group while the lowest number 48, 12% was in the age group ≥51 years.

3.1 Prevalence of Intestinal Parasites in the Study

The overall prevalence of intestinal parasites was 28.3% (113). Out of the 113 patients infected with intestinal parasites, 31.8% (87/113) were from Melong and 20.6% (26/113) from Denzo. *E. histolytica* had a prevalence of 27.7% (76/274) in Melong and Denzo 9.5% (12/126) and the difference in the prevalence of *E. histolytica* between the 2 localities was highly significant (χ^2= 16.68, P<0.001). The prevalence of *Ascaris lumbricoides* was higher in Denzo 10.3% (13/126) than in Melong 0.3% (1/274) and the difference in rate was found to be statistically highly significant (χ^2 =25.31, P<0.001) (Table 2). The prevalence of the other intestinal parasites observed can be seen in Table 2.

With respect to sex, the overall prevalence of intestinal parasites in males was 24.3% (37/152) and females 30.6% (76/248). Females were more infected with *E. histolytica* than males although the difference was not significant (χ^2 = 0.13, P = 0.720). *Trichomonas hominis* was found only in females 2.4% (6/248) with no significant difference (χ^2=3.73, P=0.05). Females were more infected with *Ascaris lumbricoides* 3.6% (9/248) than males 3.2% (5/152) but the difference was statistically insignificant (χ^2=0.32, P=0.858). *T. hominis*, *Giardia lamblia*, *Taenia spp* and *Trichuris trichiura* were not found in males (0%), while females had varied prevalence; *T. hominis* 2.4% (6/248), *G. lamblia* 1.2% (3/248), *Taenia* spp 0.4% (1/248) and *T. trichiura* 0.4%(1/248) the difference was statistically insignificant (χ^2=3.73, P=0.053; χ^2=1.85, P=0.173; χ^2 =4.27, P = 0.370; χ^2=0.61, P= 0.433), respectively. The overall prevalence of intestinal parasites was highest 35%, (21/60) in adults aged 31-50 years and the lowest 20.9% (23/110) in participants aged ≥51 years. The prevalence of *E. histolytica* was highest in adults 16-30years 27.4% (29/106) and the lowest in children ≤5 years 14.5% (16/110) but the difference was statistically insignificant (χ^2 = 5.72, P=0.221). The prevalence of *A. lumbricoides* amongst the different age group highest in children ≤ 5 years 4.5% (5/110) and lowest in patients ≥51 years 2.0 % (1/48). There was no significant difference (χ^2 =3.63, P=0.458). *Trichomonas hominis* which was least in age group ≤5 years (0%) and ≥51 years (0%) while most of the parasites was seen in 31-50 years patients (6.6%, 4/60) and the difference was statistically highly significant (χ^2=13.49, P= 0.009). *Giardia lamblia*, *Taenia* spp, *T. trichiura* had no significant difference in the prevalence amongst the various age groups (P>0.05) (Table 2).

3.2 Intensity of Intestinal Parasites in the Study Areas

Participants in Melong recorded a higher parasite load (range) 38(24-384) than those in Denzo 38(24-96) although the difference was insignificant (Mann Whitney U Test, P=0.466). In relation to sex, females, had a higher parasite load (range) 39(24-384) than males 36(24-144) but the difference was insignificant (Mann Whitney U Test, P=0.541). With respect to age, the highest parasite load (range) was recorded in patients aged 16-30 years 41(24-384) and the least in patients 31-50 years 34(24-96) and ≥51years 34(24-96) and the difference was significant (Kruskal Wallis Test, P= 0.041) (Table 3).

3.3 Prevalence and Intensity of Malaria in the Study Areas

The overall prevalence of malaria was 66.5% (266). With respect to localities, Denzo recorded a higher prevalence 79.3% (100/126) of malaria than Melong 60.5% (166/274) and the difference was highly significant ($\chi2$=13.666 P= 0.001). The prevalence of malaria was higher in males 72.3% (110/152) than in females 60.5% (166/248), although the difference was statistically insignificant (χ^2 =3.790, P=0.052). The highest prevalence of malaria, 75.4% (83/110) was recorded in the age group ≤5 years while the lowest, 56.6% (34/60) was recorded in patients aged 31-50 years and the difference was significant ($\chi2$= 12.249, P= 0.016) (Table 4).

Participants in Melong recorded a higher GMPD, 762 (160-200,000 trophozoites/µl of blood) than those in Denzo, 444 (40-10,000 trophozoites/µl of blood) and the difference was highly significant p<0.001. Females had a higher 624(40-200,000 trophozoites/µl) GMPD than males 620(40-8,000 trophozoites/µl), although the difference was insignificant (P=0.477). Based on age, the highest GMPD range was found in children ≤5 years old 645(40-200,000 trophozoites/µl of blood) and the lowest in adults ≥51years old 425(40-1,600 parasites/µl of blood), although there was insignificant difference (p=0.161) (Table 4).

Table 1. Demographic characteristics of the study population

Characteristic	Category	No. examined (%)
Site	Melong	274(68.5)
	Denzo	126(31.5)
Sex	Male	152(38)
	Female	248(62)
Age groups (Years)	≤5	110(27.5)
	6-15	76(19)
	16-30	106 (26.5)
	31-50	60 (15)
	≥51	48(12)
Total		400(100)

Table 2. Prevalence of intestinal parasites in the study areas

Parameter	Category	N	% infected (No. infected) for each parasitic species					
			***E. h* %(n)**	***A. l* %(n)**	***T. h* %(n)**	***G. l* %(n)**	**Taenia spp %(n)**	***T. t* %(n)**
Site	Melong	274	27.7(76)	0.3(1)	1.8(5)	1.0(3)	0.3(1)	0.3(1)
	Denzo	126	9.5(12)	10.3(13)	0.7(1)	0.0(0)	0.0(0)	0.0(0)
Chi-square			*16.69*	*25.31*	*0.621*	*1.39*	*0.46*	*0.46*
P-value			*<0.001*	*<0.001*	*0.431*	*0.238*	*0.497*	*0.497*
Sex	Male	152	21(32)	3.5(5)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
	Female	248	22.5(56)	3.6(9)	2.2(6)	1.2(3)	0.4(1)	0.4(1)
Chi-square			*0.13*	*0.32*	*3.73*	*1.85*	*4.27*	*0.61*
P- value			*0.720*	*0.858*	*0.053*	*0.173*	*0.370*	*0.433*
Age group (Years)	≤5	110	14.5(16)	4.5(5)	0.0(0)	1.8(2)	0.0(0)	0.0(0)
	6-15	76	23.6(18)	1.3(1)	1.3(1)	1.3(1)	1.3(1)	0.0(0)
	16-30	106	27.3(29)	2.8(3)	0.9(1)	0.0(0)	0.0(0)	0.9(1)
	31-50	60	21.6(13)	6.6(4)	6.6(4)	0.0(0)	0.0(0)	0.0(0)
	≥51	48	25(12)	2.0(1)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Chi-Square			*5.72*	*3.64*	*13.49*	*3.63*	*4.27*	*2.78*
P-value			*0.221*	*0437*	*0.009*	*0458*	*0.370*	*0.598*
Total		400	28.3(113)					

Table 3. Intensity of intestinal parasites in the study areas

Parameter		Number examined (N)	Parasite load [EPG or CPG] (Range)	Level of Significance
Site	Melong	79	38(24-384)	Mann Whitney
	Denzo	30	38(24-96)	UTest, P=0.466
Sex	Male	39	36(24-144)	
	Female	70	39(24-384)	Mann Whitney U Test, P=0.541
Age groups (Yrs)	≤5	23	47(24-144)	
	6-15	21	38(24-120)	Kruskal wallis
	16-30	33	41(24-384)	Test, P= 0.041
	31-50	18	34(24-96)	
	≥51	14	26(24-96)	
Mean EPG±SEM(range)			48±44 (24-48)	
Total		109		

EPG= Egg per gram, CPG= cyst per gram, SEM=standard error of mean

Table 4. Prevalence and intensity of malaria in the study areas

Parameter		N	n(%)	Chi-square p-value	GMPD [trophozoites/µl] (range)	P-value
Site	Melong	274	166(60.5)	χ^2=13.67	762(160-200,000)	Mann-Whitney U Test, P<* 0.001
	Denzo	126	100(79.3)	P<0.001	444(40-10,000)	
Sex	Male	152	110(72.3)	χ^2=3.79	620(40-8,000)	Mann-Whitney U test, p=0.477
	Female	248	156(62.9)	P= 0.052	624(40-200,000)	
Age groups (years)	≤5	110	83(75.4)	χ^2=12.25	645(40-200,000)	Kruskal - wallis Test, P=0.161
	6-15	76	57(75.0)	P= 0.016	827(120-8,000)	
	16-30	106	64(60.3)		578(40-40,000)	
	31-50	60	34(56.6)		557(80-4,000)	
	≥51	48	28(58.3)		425(40-1,600)	
Total		400	266(66.5)			

GMPD = Geometric mean parasite density, n= Number positive, N= Number examined, SD= standard deviation

3.4 Prevalence of Intestinal Parasite and Malaria Co-Infection in the Study Areas

The overall prevalence of intestinal parasite/ malaria co-infection was 16.3(65). Out of the 6 intestinal parasites seen in the study population, four species were co-infected with malaria (*E. histolytica, A. lumbricoides, G. lamblia and T. hominis*). The prevalence of *E. histolytica*-malaria co-infection 12.3% (49/400) was the highest and the lowest was *G. lamblia*-malaria co-infection 0.3% (1/400), Fig. 1. The highest prevalence-16.7%(21) of co-infection was found in Denzo while the lowest-16.1%(44) was found in Melong, although the difference was insignificant, p=0.878., Table 5. The highest prevalence-16.6%(41) of co-infection was seen in females while the lowest-15.8%(24) was seen in males, although the difference was insignificant, p=0.823., Table 5. The highest prevalence-20.0%(12) of co-infection was observed in the age group 31-50 years while the lowest-14.2%(15) was observed in the age group 16-30 years, although the difference was insignificant, p=0.834., Table 5.

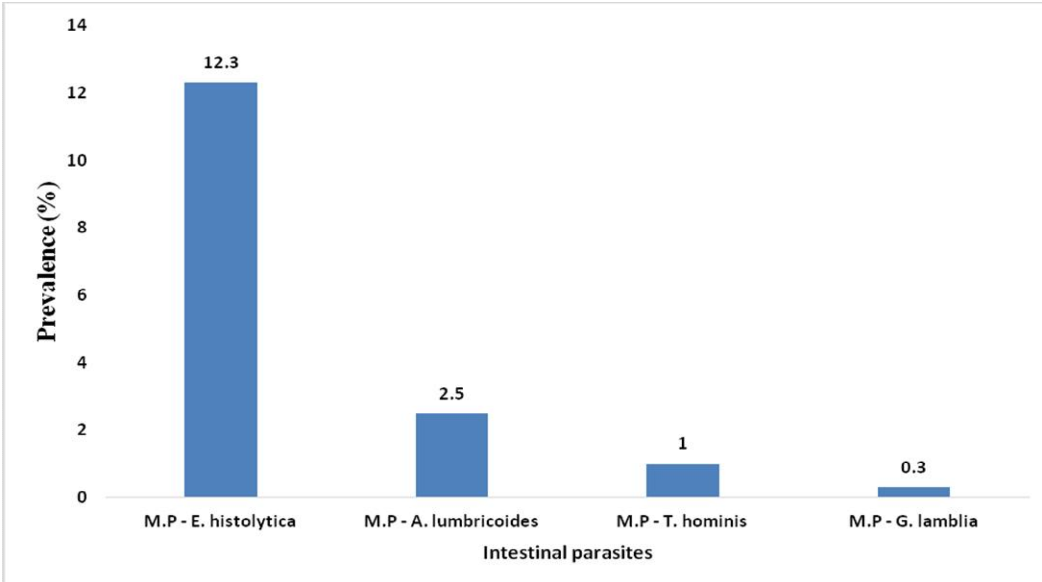

Fig. 1. Prevalence of co-infection of malaria and intestinal parasites in the study areas
(M.P= malaria parasite)

Table 5. Prevalence of intestinal parasite and malaria o-infection with respect to site, sex, and age in the study areas

Characteristic	Category	Number examined(N)	Prevalence (No. positive)	Chi-square(χ^2)	P-value
Site	Melong	274	(16.1)44	0.02	0.878
	Denzo	126	16.7(21)		
Sex	Male	152	15.8(24)	0.05	0.823
	Female	248	16.6(41)		
Age groups (Years)	≤5	110	14.5(16)	1.44	0.834
	6-15	76	17.1(13)		
	16-30	106	14.2(15)		
	31-50	60	20.0(12)		
	≥51	48	18.8(9)		
Total		400	16.3(65)		

3.5 Participants' Behaviour towards Malaria Infection

Of the 269 respondents, 82.5% (222/269) indicated that they have had malaria before and 17.5% (47/269) had never been diagnosed of malaria before. Also, the proportion of those who sleep under a mosquito bed net was 50.6% (136/269) and those who do not sleep under a mosquito bed net was 49.4% (133/269). Amongst those who sleep under a mosquito bed net, 39.8% (107/269) slept there regularly and 10.8% (29/269) slept irregularly. Following the period when they put down the bed net 0.7% (2/269) put it down when they remember, 4.8% (13/269) put it down midway in the night, 29.0% (78/269) do so when they are about to sleep and 16.0% (43/269) put it down early in the evening. The reasons given for not sleeping under a mosquito net were as follows: excess heat 15.6% (42/269), no bed Net 26.0% (70/269), no mosquitoes in the area 0.4% (1/269) and no reason 5.6% (15/269). Those who did not have their doors and windows screened were 51.3% (138/269) and those with screens on doors and windows were 48.7% (131/269). More than half of the population 71.7% (193/269) goes for confirmatory diagnosis when they have fever and joint pains and 28.3% (76/269) do not go for confirmatory diagnosis when they have fever and joint pains. However, 62.5% (168/269) take their complete malaria treatment and 37.5% (101/269) do not take their complete malaria treatment. During the evening periods, 40.5% (109/269) wear long protective clothing and 59.5% (160/269) do not. In addition, 28.3% (76/269) of the respondents had pools of stagnant water around their houses and 71.7% (193/269) did not. Amongst those who had stagnant water around their houses, 38.2% (29/269) did nothing to manage it while 61.8% (47/76) managed it by draining, Table 6.

3.6 Prevalence of Positive Behaviour towards Prevention and Control of Malaria

With respect to site, the prevalence of good behaviour was higher-56.5% (74/113) in Melong than in Denzo 42.8% (59/138) and the difference was significant (χ^2 = 5.07, p=0.024). There was a reduced chance for those in Denzo to practice good behaviour when compared to those in Melong OR=0.6(0.36-0.93) and the association was significant p=0.022, Table 7.

As far as the age was concerned, the prevalence of good behaviour was highest-58.5% (62/106) in respondents aged 21-40 years and lowest-36.8%(39/106) in respondents aged 0-20 years and the difference was significant (χ^2 =11.38, p=0.010). There was a reduced chance for respondents aged 0-20years OR=0.5(0.19-1.45) when compared with respondent aged ≥61yrs although there was no significance in the association, p=0.210. Respondents within the age 21-40years 58.5% (62/106) and 41-60years 57.5% (23/40) had an increased chance of practicing good behaviour OR=1.3(0.45-3.50) and OR=1.2(0.39-3.76), respectively although there was no significant difference in the association (p= 0.668 and p=0.751 respectively), Table 7.

Table 6. Participants' behaviour towards malaria infection

Questions	Response	Frequency %(N)
Have you ever been diagnosed of malaria before?	No	17.5(47)
	Yes	82.5(222)
Do you have a mosquito net?	No	49.4(133)
	Yes	50.6(136)
If yes how often do you sleep under a mosquito net?	Don't use a mosquito net	49.4(133)
	irregularly	10.8(29)
	regularly	39.8(107)
When do you put down your mosquito net?	Don't have	49.4(133)
	When I remember to put it down	0.7(2)
	Mid way in the night	4.8(13)
	When about to sleep	29.0(78)
	Early in the evening	16.0(43)
Why don't you sleep under a mosquito net?	Difficulties in breathing	1.9(5)
	Excess heat	15.6(42)
	No bed net	26.0(70)
	No mosquitoes in my area	0.4(1)
	No reason	5.6(15)
	Users	50.6(136)
Do you have screens on doors and windows of your house?	No	51.3(138)
	Yes	48.7(131)
Do you usually go for confirmatory diagnosis when you have fever or joint pain?	No	28.3(76)
	Yes	71.7(193)
Do you usually take your complete malaria treatment?	No	37.5(101)
	Yes	62.5(168)
Do you usually wear long protective clothing during the evening period?	No	59.5(160)
	Yes	40.5(109)
Are there pools of stagnant water around your house?	No	71.7(193)
	Yes	28.3(76)
How do you manage them?	Nothing	38.2(29)
	Draining	61.8(47)

According to the occupation, civil servants recorded the highest prevalence 72.4% (21/29) of good practices towards malaria prevention and control and the lowest was recorded in those unemployed 38.2%(47/105). The results of the disparity was highly significant (χ^2 =14.27, p=0.003). The unemployed and farmers had a reduced chance of practicing good behaviour OR=0.5(0.26-0.83); 0.8(0.39-1.83) respectively when compared to traders. The association with unemployed was statistically highly significant. P=0.009 but not significant with farmers P=0.148. Civil servants had an increased chance of practicing good behaviour OR=2.0(0.78-5.02) although the association was not significant P>0.05.

Furthermore, with respect to the level of education, tertiary respondents had the highest prevalence 55.0% (22/40) and the lowest prevalence in people with no formal education 36.4% (4/11), the difference was not significant P=0.605. There was a decreased chance of respondents with no formal education OR=0.5(0.12-1.85), primary education OR=0.9(0.42-2.02) and secondary education OR=0.7(0.37-1.48) of practicing good behaviour when compared to those with tertiary education although the associations were not significant (P=0.279, P=0.836, P=0.390 respectively), Table 7.

3.7 Participants' Habits towards Intestinal Parasites Infection and Drinking Water Source

Based on the questionnaire findings on the habit of the people in the two study location towards intestinal parasite infection, the number of people that had ever been diagnosed of intestinal parasites was higher 58.4%, (157/269) than those who had never been diagnosed of intestinal parasites 41.6% (112/269). The hygiene modality of hand washing was higher in those who washed their hands 87.4%, (235/269) than those who do not wash their hands 12.6% (34/269). Amongst those who washed their hands, the frequency of those who use water and soap was higher 47.6% (128/269) than those who washed with water only 39.8% (107/269), Table 8. Those who practiced hand washing behaviour after defecation were 87.7% (236/269) while only 12.3% (33/269) did not wash their hands after defecation. Also, 86.6% (233/269), of respondents practiced washing of fruits and vegetables before eating while 13.4 (36/269) did not. The majority of the people used latrine 72.1% (194/269) while the minority used the water system 27.97% 75/269). Almost half of the indigenes of the study population used tap water as their source of drinking water (49.8%, 134/269), followed by the borehole source 26.4% (71/269), then protected well 14.9% (40/269)and streams 8.9% (24/269) being the least used source of drinking water, Table 8.

3.8 Patients' Behaviour towards Prevention and Control of Intestinal Parasites

In general, the prevalence of good behaviour was higher in Melong 69.5% (91/131) than in Denzo 56.5% (78/138) and there was a significant difference (χ2=4.821, P=0.028). The people of Denzo were less likely to practice good behaviour when compared with the people of Melong, and the association was statistically significant P =0.029, Table 9.

In relation to age, respondents ≥61 years old had the highest prevalence of good behaviour 88.2% (15/17) and the lowest was in respondents aged 0-20 years 57.5% (61/106) although the difference was insignificant (χ2=6.34, P=0.096). The likelihood of respondents aged 0-20 years; OR=0.2(0.04-0.83), 21-40 years OR=0.2(0.05-1.15) and 41-60 years OR=0.2(0.04-0.99) to practice good behaviour compared to those aged ≥61years was reduced and the associations were significant (p=0.028, p=0.05) except for those aged 21-40 years; P=0.074, Table 9.

In the relation to gender, females had a higher prevalence 65.2% (107/164) of good behaviour than males 59.0% (62/105) although the difference was insignificant. There was a reduced chance for males to practice good behaviour when compared to females; OR=0.8(0.46-1.27) but this relation was insignificant at P= 0.305, Table 9.

With respect to occupation, the highest prevalence of good practice was recorded incivil servants 89.7% (26/29) and the lowest in farmers 52.6% (20/38) with a statistical highly significant difference (χ2=11.81, P=0.008). The possibility of unemployed and civil servant to practice good behaviour was increased when compared to those of traders, which was highly significant for civil servants (P = 0.004) and insignificant in unemployed (P = 0.360). Farmers had a reduced chance of practicing good behaviour when compared to traders OR=0.8(0.39-1.83) but the relation was not significant, Table 9.

Table 7. Prevalence of positive behaviour towards prevention and control of malaria in the study areas

Parameter		Number Examined	Prevalence of Good Behaviour	Significance	Binary Logistic Regression or (Ci)	P-Value
Site	Melong	131	56.5%(74)	$\chi^2 = 5.07$	reference	-
	Denzo	138	42.8%(59)	p=0.024	0.6(0.355-0.932)	0.025
Age group (years)	0-20	106	36.8%(39)	$\chi^2 = 11.38$	0.5(0.185-1.451)	0.210
	21-40	106	58.5%(62)	p=0.010	1.3(0.448-3.501)	0.668
	41-60	40	57.5%(23)		1.2(0.385-3.761)	0.751
	≥61	17	52.9%(9)		reference	
Sex	Males	105	35.2%(37)	$\chi^2 = 13.90$	0.4(0.232-0.640)	<0.001
	Females	164	58.5%(96)	p<0.001	reference	-
Occupation	Unemployed	123	38.2%(47)	$\chi^2 = 14.27$	0.5(0.263-0.830)	0.009
	Civil servants	29	72.4%(21)	p=0.003	2.0(0.784-5.017)	0.148
	Farmers	38	52.6%(20)		0.8(0.386-1.826)	0.659
	Traders	79	57.0(45)		reference	-
Level of Education	No formal	11	36.4%(4)	$\chi^2 = 1.85$	0.5(0.118-1.854)	0.279
	Primary	68	52.9%(36)	p=0.605	0.9(0.420-2.016)	0.836
	Secondary	150	47.3%(71)		0.7(0.365-1.482)	0.390
	tertiary	40	55.0%(22)		reference	-

Table 8. Participants' habit towards intestinal parasites infection

Questions	Response	Frequency (N)
Have you been diagnosed of intestinal parasite before?	No	41.6(112)
	Yes	58.4(157)
Do you wash your hands before eating?	No	12.6(34)
	Yes	87.4(235)
If yes what do you use	Don't wash hands	12.6(34)
	Water only	39.8(107)
	Water and soap	47.6(128)
Do you wash your hands after using the toilet?	No	12.3(33)
	Yes	87.7(236)
Do you wash your fruits and vegetables before eating?	No	13.4(36)
	Yes	86.6(233)
What type of toilet system do you have?	Latrine	72.1(194)
	Water system	27.9(75)
What is your source of drinking water	Stream	8.9(24)
	Bore hole	26.4(71)
	Protected well	14.9(40)
	Tap water	49.8(134)

Table 9. Prevalence of positive behaviour towards prevention and control of intestinal parasites in the study areas

Parameter	Indicators	Number examined	Prevalence of good behaviour	Chi-square, P-value	Binary logistic regression OR (CI)	P-value
Site	Melong	131	69.5%(91)	$\chi^2 = 4.82$	Reference	0.029
	Denzo	138	56.5%(78)	p=0.028	0.6(0.346-0.944)	
Age group (years)	0-20	106	57.5%(61)	$\chi^2 = 6.34$	0.2(0.039-0.830)	0.028
	21-40	106	65.1%(69)	p=0.096	0.2(0.054-1.147)	0.074
	41-60	40	60.0%(24)		0.2(0.040-0.996)	0.049
	≥61	17	88.2%(15)		reference	-
Sex	Males	105	59.0%(62)	$\chi^2 = 1.05$	0.8(0.464-1.272)	0.305
	Females	164	65.2%(107)	p=0.305	reference	-
Occupation	Unemployed	123	63.4%(78)	$\chi^2 = 11.81$	1.3(0.735-2.332)	0.360
	Civil servants	29	89.7%(26)	p=0.008	6.5(1.829-23.443)	0.004
	Farmers	38	52.6%(20)		0.8(0.386-1.826)	0.659
	Traders	79	57.0%(45)		reference	-
Level of Education	Unknown	11	45.5%(5)	$\chi^2 = 8.82$	0.2(0.42-0.746)	0.018
	Primary	68	58.8%(40)	p=0.032	0.3(0.117-0.782)	0.014
	Secondary	150	60.7%(91)		0.3(0.136-0.788)	0.013
	Tertiary	40	82.5%(33)		reference	-

4. DISCUSSION

Our study sought to determine the prevalence rate of intestinal parasites/malaria co-infections and their associated risk factors in Melong and Denzo, Littoral region- Cameroon. Our findings are explained below.

The overall prevalence of intestinal parasites was 28.8% and it varied with the location. This was lower compared to what was reported by Fusi-Ngwa et al. [16] in Dschang, a locality of West region of Cameroon where the prevalence of intestinal parasites was 34.7% and Omar *et al*. [17] who recorded intestinal parasites prevalence of 45.38% in Northwestern Saudi Arabia. The prevalence of intestinal parasites was higher in Melong, the semi-urban area than in Denzo, rural area. Although these infections are usually associated with poor sanitary habits, lack of access to safe water and improper hygiene which is more prominent in rural areas. Melong is at a cross road to most major towns where travelers usually stop to take a bite and consume different types of food which can encourage the propagation of intestinal parasites in this area than in Denzo, which is far off. This was in contrast to a study carried out by Kimbi *et al.* [18] with higher prevalence in rural area than in urban area due to urbanization.

Entamoeba histolytica had the highest prevalence amongst the intestinal parasites, with the prevalence of *Entamoeba histolytica* in Melong being significantly higher than that in Denzo (P<0.001). *Entamoeba histolytica* is an indicator organism of faecal contaminated food or water and it is mostly present in unhygienically prepared food which leads to the outbreak of amoebiasis. The higher prevalence in Melong might be because of the high level of consumption of fish and meat along the road, which is likely to be undercooked or contaminated with faecal matter or the consumption of contaminated water. Also, semi-urban markets might sell contaminated vegetables which are sometimes eaten raw, undercooked to retain taste and preserve heat-labile nutrients. It could also be due to the presence of resistant cysts in Melong. *Ascaris lumbricoides* was significantly higher in Denzo than in Melong P<0.001. This difference might be because; *Ascaris lumbricoides* is a soil-transmitted helminth and can be acquired by ingesting its eggs in contaminated food or rarely water. Most of the people of Denzo are farmers and this might have increased their chances of ingesting the egg directly from soil via dirty hands. This was similar to what was observed by Kimbi *et al.* [18] in the Mount Cameroon region, with higher prevalence of *A. lumbricoides* due to lower level of sanitation in the rural areas. *Entamoeba histolytica* was highest in adults aged 16-30 years although the difference was not significant (P=0.221). This could be because, this age group 16-30 years are the most active working group, some could be food handlers who do not take hand washing seriously, their level of education especially on knowledge of transmission of amoebiasis is low and they are most likely to keep long finger nails which will serve as a transmission medium. The findings of this study were in contrast with that observed by Zaglool et al. [19] where children less than five years of age had the highest prevalence of *E. histolytica* due to poor hand hygiene in both children and caregivers and lack of portable water supply. It was higher compared to studies done by Banke *et al*. [20], Akingbade *et al.* [21] and Ismail, [22] who recorded 7.06%, 19.4% and 2%, respectively.

Trichomonas hominis was higher in adult age 31-50 years and least in children ≤5years and ≤51 years and the difference was significant (P=0.009). This is a zoonotic infection that can be transmitted through the faeco-oral route or from formites and adults are more exposed as they play with animals than children. Also, adults are engaged with farm activity more which exposes them to fecal matter. This is in contrast with an experiment carried out by Inoue *et al.* [23] on marmosets where the infection of *Trichomonas hominis* was not due to age or sex.

According to the results of this survey, it was noticed that people of Melong and Denzo localities of Cameroon are more infected by malaria than intestinal parasites. This can be explained by the failure to prevent malaria infection in the two localities. The overall prevalence of malaria was 66.5% which was higher than that reported by Nkuo-Akenji et al. [24] in Mount Cameroon region and Oboth et al. [25] in Mid Western Uganda, which was 64% and 55.04%, respectively. This indicates that the prevalence of malaria in Cameroon is still high despite the intermittent prevention and control measures put in place. Our study reveals that the prevalence of malaria was higher in Denzo (rural area) than in Melong (semi-urban) and the difference was highly significant (P<0.001). The higher prevalence in the rural area could be due to greater risk of vector contact and infection as a result of no bed nets, most of them lack screens on doors and windows of their houses, poor living conditions, stagnant water and bushes around the house. Also, the high prevalence could be due to insufficient knowledge and practice of good behaviour towards malaria prevention and control measures. These results are similar to study carried out by Kimbi *et al*. [18] and M'bondoukwe et al. [26] who also noticed that rural areas have a higher prevalence than semi-urban and urban areas because their houses were made of plank with crevices on the walls.

This study revealed that that the prevalence of malaria in males was higher than in females, although the difference was insignificant (P=0.052). This could probably be because males are more exposed to the malaria vector than females as they work in the fields at peak biting time or move to malarious areas for work. This finding was similar to that in studies carried out by Das et al. [27] and Yusof et al. [28] who also noticed higher prevalence of malaria in males than in females due to the nature of their jobs as they are mainly into agriculture such as farming, forestry workers and loggers, who have higher risk of exposure to the *Anopheles* mosquitoes.

The prevalence of malaria varied significantly (P=0.016) with age with children aged ≤5 years having the highest prevalence. This might be because young children are more vulnerable to malaria parasite infection as they have gotten little or no partial immunity to the parasite. The severity of the attack of *Plasmodium* spp depends on circumstances such as the state of immunity and the general health and nutritional status of the infected individuals. Older patients have probably had several attacks of malaria which help them develop acquired immunity that can protect them from severe attacks or death [29]. Although no one develops complete immunity against malaria that can fully protect the person from infection.

The overall prevalence of co-infection was 16.3%. The prevalence of co-infection in Denzo was higher than in Melong; the risk factors associated to single infection might have been the same reason for co-infection. This was in contrast with what was reported by Shapiro et al. [30] in a study conducted in Uganda which showed no association of malaria with intestinal parasites. This was probably because in areas of frequent exposure to malaria, immunity is developed and so the effect of helminthes co-infection was minimal when transmission intensity is low. Also, the disparity might have been due to the genetic makeup of individuals, which may confound the nature of relationship between malaria parasite and intestinal parasites [31].

Malaria is commonly found in the warmer regions of the world that are closer to the equator including, tropical and subtropical countries. Also, low altitudinal areas favour the prevalence of malaria than high altitudinal areas [32]. Malaria parasite which develops in the mosquito, also require warm environment before reaching the stage at which they are ready to be transmitted to humans [33]. Also stagnant water that serves as breeding grounds for larval forms of malaria vector, thick bushes that provides hiding places for mosquitoes, hence their survival [34].

In this study the prevalence of good behaviour towards malaria practices was higher in adults aged ≥61years and lower in those 0-20 years and the difference was significant (P=0.010). This could be because children in this age group are likely not to take good practices towards malaria seriously such as wearing of protective clothing, seeing the need to sleep under mosquito treated bed nets. The findings of this study corroborates the finding of a study carried out by Sixpence *et al*. [35] which showed that people with low levels of malaria knowledge are greater than two times more likely to experience poor health outcomes.

Behavioural risk factors of malaria in relation to sex indicates that females practice good behaviour toward malaria prevention and control than males with a significant difference (P<0.001). This is similar to the study conducted by Mbenda et al. [36]. The reason for this could be that, males are breadwinners and because of that role they may stay longer outside in the night looking for means to cater for their families predisposing them to mosquito bites. Again, the nature of their occupation, as they mainly belong to the agricultural groups including farming , forestry site workers, and loggers who have a higher risk of exposure to the *Anopheles* spp mosquitoes [27,28].

With respect to occupation, civil servants had the highest prevalence of good behaviour and the lowest in unemployed (38.2%) (P=0.003). This study contradicts the result obtained by Ramdzan et al. [37] where most of the people employed were mainly infected with malaria. The reason for the unemployed having lower prevalence of good practice toward malaria prevention and control could be due to lack of finances, whereby they might not be able to get protective clothing, screens on doors and windows of their houses or go to the hospital for confirmatory diagnosis or able to buy drugs for malaria treatment.

According to the level of education, those with tertiary education had the highest prevalence of good behaviour and the lowest in those with no formal education although the difference was not significant. This could be because those with no formal education are ignorant about malaria prevention and control measures. This finding is in line with one study carried out in Nigeria which demonstrated that higher levels of education were associated with improved knowledge and practice about appropriate malaria prevention and control interventions. Education also increases the probability that households would purchase insecticide treated bed nets [38].

The socio-economic situation of individuals in different countries following epidemiological studies has been shown to be most important factor in the prevalence of intestinal parasites. Factors from unhygienic habits, contaminated food and water to poor sanitary disposal. Ignorance and illiteracy can also be attributed to these infections due to lack of information on the mode of transmission and symptoms of intestinal parasites [39].

In relation to site, Melong had good practice toward intestinal parasite prevention and control than those in Denzo and the difference was significant. The association for them to practice good altitude when compared to Melong was reduced P=0.029. This could be because urbanization in Melong helps to reduce the chance of bad practices compared to Denzo (rural area), where there is farming activities, and low–economic status.

Adults aged ≥61 years have the highest prevalence of good practice towards intestinal parasites and lowest within the age 0-20 years although the difference was not significance. Amongst those who responded not to wash hands before eating and after the toilet, majority of them were children. This could partly be explained by the lack of adequate facilities and soaps in the toilets for use at home or due to the fact that children catch germs when they touch contaminated objects or surfaces or soil which increases the risk of hand contamination with disease causing pathogens. Handwashing is the single most effective way to prevent the spread of infections [40].

With respect to occupation, civil servants had the highest prevalence of good behaviour towards intestinal parasites and the lowest were farmers with a significant difference. This could be because most farmers walk and work barefooted in the farm and usually eat in the farm without properly washing their hands, or eat raw food which may be contaminated. Our findings are in line with a study in carried out in Uganda which revealed that farmers are particularly vulnerable to infections with intestinal parasites, soil transmitted helminths and *Schistosoma mansoni* because most of them live within marginalized communities with little or no integrated sanitation safety measures being practiced [41].

In general, poor drinking water source, lack of personal and community hygiene, presence of bushes and water bodies are the most confounding risk factors leading to the cause of co-infection in these localities.

5. CONCLUSIONS

This study concluded that the overall prevalence of intestinal parasites in Melong was 31.7% and in Denzo 20.6% and the overall prevalence of malaria in Melong was 60.5% and Denzo was 79.3%. This study showed that the prevalence of co-infection of malaria-*Entamoeba histolytica* was the highest co-infection in the study area and the overall prevalence of co-infection malaria-intestinal parasites was 16.3%. Low access to bed nets, presence of bushes and water bodies, poor drinking water source and lack of personal and community hygiene are the associated risk factors leading to co-infection of malaria and intestinal parasites. Despite the intestinal parasites / malaria measures put in place to effectively prevent and control these diseases, they are still a cause of morbidity and mortality in Cameroon.

Therefore, we recommend that interventions should thus, target children who are at risk, in order to reduce high prevalence in Melong and Denzo, littoral region, Cameroon. The Ministry of Public Health should therefore consider the re-distribution of insecticide treated bed nets and sensitised the people on the importance of using the bed nets which will help reduce the risk of malaria as most of the people complained of no bed nets. There is a need of the Ministry of Public Health to institute some control measures such as intermittent preventive treatment of malaria and intestinal parasites in Melong and Denzo, Cameroon.

CONSENT AND ETHICAL APPROVAL

An ethical clearance was obtained from the University of Bamenda review board. Administrative clearances to carry out this study in Melong district hospital and Denzo integrated Health centre were obtained from the Director of the respective medical facilities. Participation in this study was voluntary. Participants had to fill and sign the informed consent form which explained the benefits of the study (that is, those found infected would be treated). Parents/legal guardians had to fill and sign the informed consent form for minors (children below 18 years).

EXCLUSION CRITERIA

Patients who did not present stool samples for analysis and blood for malaria parasite examination in Melong District Hospital and Denzo integrated Health Centre were excluded. Patients who did not bring the required quantity of stool sample and those who did not sign the informed consent form were excluded. Those who signed the informed consent form but wanted to withdraw were not forced to participate in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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