

Multiparasitism among Schoolchildren of Akonolinga, Nyong et Mfoumou Division, Centre Region of Cameroon

Martin Gael Oyono (Corresponding author)

Laboratory of Parasitology and Ecology, Faculty of Sciences, University of Yaoundé I,

P.O Box: 812 University of Yaoundé I, Yaoundé-Cameroon

Email: oyono.martingael@gmail.com

Leopold Gustave Lehman

Parasitology and Entomology Unit, Laboratory of Animal Biology and Physiology,
Faculty of Sciences, University of Douala, P.O. Box: 2701 University of Douala,
Douala-Cameroon

Email: leopoldlehman@gmail.com

Samuel Fosso

Laboratory of Parasitology, Mycology and Parasitic Immunology, Faculty of Medicine,
and Biomedical Sciences, University of Yaoundé I, P.O. Box: 1364 Yaoundé Cameroon

Email: fossosamuel@yahoo.fr

Charles Félix Bilong Bilong

Laboratory of Parasitology and Ecology, Faculty of Sciences, University of Yaoundé I,

P.O Box: 812 University of Yaoundé I, Yaoundé-Cameroon

Email: bilong_bilong@yahoo.com

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Abstract

In general, school-age children are the most vulnerable to parasitic infections and are particularly exposed to multi-parasitism and its potential consequences. This study aimed at determining the intensity of multi-parasitism in Nyong et Mfoumou Division. A cross-sectional study took place from September 2017 to July 2018 among pupils of five (05)

government schools from the Nyong et Mfoumou Division. Stool samples were collected from each child and examined for protozoan cysts, helminth eggs and larva while blood samples were collected for detection of *Plasmodium sp.* and filarial blood stages. In addition, socio-demographic information were documented. In total, 416 schoolchildren were recruited; out of which 309 (74.28%) were infected by at least one parasite species. 13 parasite species were found: 03 blood parasites and 10 intestinal parasites. *Plasmodium falciparum* was the main blood parasite (37.26%). Amongst intestinal parasites, *Entamoeba coli* were the most common among protozoa (29.33%) and *Ascaris lumbricoides* among helminths (21.39%). The frequency of multi-parasitism was 44.47% and the average species reach was 1.43 ± 0.01 per individual. Four types of multi-parasitism were found (bi-parasitism, tri-parasitism, quadri-parasitism and penta-parasitism); the bi-parasitism (26.68%) was the most common. Significantly statistic associations were found between parasite species such as: *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Ascaris lumbricoides*, *Trichuris trichiura* can be explained by the same means of transmission. Association between *Ascaris lumbricoides* and *Mansonella perstans* could be a synergic interaction between these parasites. We conclude that the intensity of multiparasitism among schoolchildren in Nyong et Mfoumou Division is high with predominance in rural areas.

Keywords: multiparasitism, frequency, determinants, parasitic association, schoolchildren

1. Introduction

More than 80% of all living species described to date are parasites (Vaumourin *et al.*, 2015). They are great in diversity and parasitize a wide range of hosts they often share together. The concomitant presence of two or more parasite species in the same host, called multi-parasitism, appears as the rule than the exception in most biological systems including humans (Petney & Andrews, 1988).

In infected zones, more than 30% of infections are multiparasitism and this rate can reach up to 80% in some human populations (Petney & Andrews, 1988). Co-infective parasites interact directly or indirectly through several interspecific mechanisms. These interactions can affect the host's health because they modify a large number of factors including the host's susceptibility to other parasites, duration of infection, risks of transmission, clinical signs, therapeutic success and control strategies (Vaumourin *et al.*, 2015).

Multiparasitism results either from synergistic interactions between two or more parasite species infecting the same host or from a community of risk factors between these parasites, which thus creates statistical associations between them. These factors may be environmental, climatic, related to the host's behavior and physiological conditions, and the transmission means of these parasites (Lello *et al.*, 2013; Vaumourin *et al.*, 2015).

In the Nyong et Mfoumou Division, populations are predominantly rural. They have limited access to safe water, sanitation and basic health services. In addition, climatic and environmental conditions are favorable for the development and persistence of several parasite species. Everywhere in sub-Saharan Africa, school-age children are the most vulnerable segment of the population to parasitic infections, especially intestinal and malaria

parasites because of behavioral, hygienic and recreational reasons (Hamit *et al.*, 2013). School-age children infected with intestinal helminths undergo frequent physical and mental sufferings due to anemia, which result in a lack of attention, inability to assimilate knowledge and contribute to absenteeism and school dropouts. Intestinal worms are also responsible for a decreased immunity of children towards malaria (Hamit *et al.*, 2013). In addition, these children are thus most exposed to multiparasitism and its potential consequences (Brooker *et al.*, 2006). Little is known on the frequency and intensity of multiparasitism in Cameroon especially in the Akonolinga area. We therefore conducted a study with the aim to determine the frequency of multiparasitism, look for its determinants in Nyong et Mfoumou Division in the Centre Region of Cameroon and study parasitic associations among species.

2. Material and Methods

2.1 Study Area

We conducted a cross-sectional study from September 2017 to July 2018 in Akonolinga, the capital of Nyong et Mfoumou Division in the Centre Region of Cameroon. This division covers an area of 4,300 Km² approximately with a bit more than 100,000 inhabitants (Bayaga *et al.*, 2017). The climate is typically equatorial with two discontinuous dry and wet seasons. The annual average rainfall is 2000 mm with an annual average temperature of 24 °C (Zeukeng *et al.*, 2014). The hydrographic network is dense with two main rivers: Nyong and Mfoumou. Several economic activities are developed consisting mainly of agriculture, livestock, fishing, hunting and small businesses. Houses are built in semi-dur with crevices and open joints serving as hideouts for mosquitoes. These villages lack access to potable water. Toilet facilities, made up essentially of pit latrines, are in general poorly constructed and insufficient for the members of a household.

Several prospecting trips were organized on the study area. In order to recruit at least 250 participants in each living areas, we randomly selected five (05) Government schools: 3 in rural areas and 2 in urban areas for participant's recruitment.

2.2 Ethical Considerations

This study was approved by the National Ethical Committee of Research for Human Health (Ethical Clearance N^o: 2018/01/968/CE/CNERSH/SP) and the Direction of Yaoundé University Teaching Hospital (Research Authorization N^o: 894/AR/CHUY/DG/DGA/DMT). However, written informed consent was obtained from parents or legal guardians of all children prior to their inclusion in the study.

2.3 Sample Collection

Before sample collection, socio-demographic information (gender, age, class, living area and school's name) of each child data and school environment related information were collected with semi-structured questionnaire. Afterwards, each child was given a sterile and labeled stool container as well as instructions for the adequate collection of their stools. After collection, stool samples were fixed *in situ* with formalin solution diluted to 10%. Blood samples were collected from each child by pricking finger between 10:00 am and 4:00 pm for maximizing the chance of detection blood filarial. Three drops of blood were collected to realize two calibrated thick and one thin blood films which were then air-dried, stored in slide boxes and transported to the Laboratory of Parasitology, Mycology and Parasitic Immunology of the Yaoundé University Teaching Hospital for parasitological examinations.

2.4 Parasitological Examinations

Each stool sample was screened by direct examination (2 slides for each stool sample) and Formalin-ether concentration technique (at least 2 slides for each stool sample) (Uga *et al.*, 2010) for the presence of protozoa cysts and helminths eggs and larva. For each participant, two calibrated thick and one thin blood films were stained with May-Grünwald-Giemsa (WHO, 2010) and examined for the presence of *Plasmodium sp.* and filarial blood stages. All slides were read using the CyScope® microscope (Partec-Sysmex GmbH, Görlitz, Germany) in a blind manner by two qualified technicians. In case of discrepancy, a third qualified technician was called to read the quarreled slides. For blood smears, slides were negative in case of absence of any trophozoite after examination of at least 10 fields for *Plasmodium sp.* or absence of microfilaria in entire slide for blood filarial. The entire slides were examined for each stool slide and if any parasite forms was not observed, slides were reported negative.

2.5 Statistical Analysis

Data was keyed in a Microsoft Excels 2007 spreadsheet then exported to SPSS 16.0 (SPSS, Chicago, Inc., IL, USA) software for statistical analysis. Frequencies of socio-demographic data of participants, and the presence of parasites species were determined. To compare single parasite infections by gender, age groups and living areas, χ^2 -test was used to compare proportions. The frequency of multiparasitism was assessed and stratified by gender, age groups and living areas. Multivariable logistic regression was used to investigate associations between parasites and socio-demographic data. *P-value* less than 0.05 were considered statistically significant.

3. Results

3.1 Study Population

In total, 416 schoolchildren, 209 from rural area and 207 from urban area, were included in the study. Females accounted for 54.33% (n = 226) of all participants giving a male-to-female sex ratio of 0.8. In addition, 117 and 109 females were reported in rural and urban areas respectively. The age of children ranged from 4 to 15 years with a mean value of 9.17 ± 0.27 years. Children were grouped into 3 age sub-groups of 4 years interval (Table 1). Gender-distribution was similar with respect to these three age groups ($\chi^2 = 1.49$; $P = 0.47$). Children aged from 4 to 7 years and from 12 to 15 years be more frequent in urban areas with 68 and 76 children against 67 and 36 children in rural areas respectively. This pattern was inverted among those aged between 8-11 years where they were more frequent in rural areas than in urban ones (106 versus 63).

Table 1. General characteristics of the study population

	Total population (N = 416)	Rural area (n = 209)			Urban area (n=207)	
		GS Essang-Ndibi (n=73)	GS Kpwele (n=72)	GS Eboa (n=64)	EPA (n=76)	GS Loum (n=131)
Gender %(n)						
Male	45.67 (190)	45.21 (33)	50.00 (36)	35.94(23)	38.16(29)	52.67(69)
Female	54.33 (226)	54.79 (40)	50.00 (36)	64.06(41)	61.84(47)	47.33 (62)
Age sub-group (years) %(n)						
4 – 7	32.45(135)	41.09 (30)	26.39 (19)	28.12(18)	32.89(25)	32.82 (43)
8 – 11	40.63 (169)	34.25 (25)	62.50(45)	56.25(36)	42.11(32)	23.67 (31)
12 – 15	26.92 (112)	24.66 (18)	11.11 (8)	15.63(10)	25.00(19)	43.51 (57)

N: Total population, n: sub-population, GS: Government School

3.2 Frequencies of Parasite Species Among Pupils

Out of the 416 samples examined, 309 (74.28%) were infected with at least one parasite species. Thirteen (13) different parasite taxa including 3 blood parasites and 10 intestinal parasites (6 protozoa and 4 helminths) were recorded. One hundred and sixty-seven (167) individuals were infected with blood parasites (40.14%) and 250 (60.10%) with intestinal parasites. Table 2 below summarizes frequencies of different group and parasite species related to total population with regard to age groups, living area and gender. Pupils living in rural areas were more infected with blood parasites ($P = 0.0047$) and intestinal parasites ($P = 0.0000$) than those living in urban areas.

Plasmodium falciparum was the most common blood parasites followed by *Mansonella perstans* and *Loa loa*. More than one third (37.26%) of participants had an infection with *Plasmodium falciparum*. This infection was not influenced by age group nor gender but was significantly ($P = 0.0139$) higher in rural areas than in urban areas. The overall prevalence of *Mansonella perstans* was 4.32 % and likewise was not influenced by age group nor gender as *Plasmodium* infection. *Loa loa* was found only in rural areas, with infection prevalence of 0.48%.

The prevalence of intestinal helminths was significantly higher in rural areas (51.19%, $P < 0.0001$) and females (37.61%, $P = 0.018$) compared to urban areas (13.52%) and males (26.31%). The highest infection rate among this group of parasites were found for *Ascaris lumbricoides* (21.39%); and its prevalence was also found to be higher in pupils of rural areas (33.49%, $P < 0.0001$) and females (25.22%, $P = 0.018$). Likewise, a high infection rate was reported for *Trichuris trichiura* with a value of 18.51%. Its prevalence was significantly higher in rural areas than in urban areas ($P < 0.001$); participants' age did not influence the risk for infection with these both parasites ($P > 0.05$). Hookworm and *Hymenolepis nana* were also found in this study with prevalence of 0.96% and 0.24% respectively (Table 2).

Entamoeba coli was the main intestinal protozoa reported in this study (29.33%) and its prevalence was significantly higher in rural areas (42.58% versus 15.9%; $P = 0.0001$). The overall prevalence of *Entamoeba histolytica/dispar* was 23.80% and was significantly higher among schoolchildren aged from 12 to 15 years ($P = 0.042$). Prevalence of *Giardia intestinalis*, *Endolimax nana* and *Blastocystis sp.* were 4.09%, 3.13% and 1.44% respectively. *Embadomonas intestinalis* was found only in rural areas with a prevalence of 0.24% (Table 2).

Table 2. Frequency of different parasite groups and species related to Total population, age groups, living area and gender

Groups of parasites	Total Population N (%)	Age groups			P	Area			P	Gender		
		4 – 7 n(%)	8 – 11 n(%)	12 – 15 n(%)		Rural n(%)	Urban n(%)	M n(%)		F n(%)	P	
Hemoparasites												
<i>P. falciparum</i>	155 (37.26)	42 (31.11)	72 (42.60)	41 (36.60)	0.1184	90 (43.06)	65 (31.40)	0.0139*	73 (38.42)	82 (36.28)	0.6532	
<i>M. perstans</i>	18 (4.32)	07 (5.18)	06 (3.55)	05 (4.46)	0.7821	13 (6.22)	05 (2.41)	0.0565	12 (6.31)	06 (2.65)	0.0675	
<i>L. loa</i>	02 (0.48)	00 (0.00)	02 (1.18)	00 (0.00)	/	02 (0.95)	00 (0.00)	/	01 (0.52)	01 (0.44)	/	
Intestinal Parasites												
Protozoa												
<i>E. coli</i>	122 (29.32)	42 (31.11)	51 (30.17)	29 (25.89)	0.6365	89 (42.58)	33 (15.94)	0.0001*	48 (25.26)	74 (32.74)	0.095	
<i>E.histolytica/dispar</i>	99 (23.79)	22 (16.29)	45 (26.62)	32 (28.57)	0.042*	46 (22.00)	53 (25.60)	0.389	38 (20.00)	61 (26.99)	0.095	
<i>G. intestinalis</i>	17 (4.08)	04 (2.96)	10 (5.91)	03 (2.67)	0.2943	13 (6.22)	04 (1.93)	0.0272*	13 (6.84)	04 (1.76)	0.027*	
<i>Blastocystis sp.</i>	06 (1.44)	03 (2.22)	01 (0.59)	02 (1.78)	0.4652	06 (2.87)	00 (0.00)	0.014*	02 (1.05)	04 (1.76)	0.541	
<i>Em. intestinalis</i>	01 (0.24)	01 (0.74)	00 (0.00)	00 (0.00)	/	01 (0.47)	00 (0.00)	/	01 (0.52)	00 (0.00)	/	
<i>En. nana</i>	13 (3.12)	06 (4.44)	04 (2.36)	03 (2.67)	0.5568	08 (3.82)	05 (2.41)	0.407	07 (3.68)	06 (2.65)	0.547	
Helminths												
<i>lumbricoides</i>	89 (21.39)	23 (17.04)	42 (24.85)	24 (21.42)	0.2559	70 (33.49)	19 (9.17)	0.0000*	32 (16.84)	57 (25.22)	0.0379*	
<i>T. trichiura</i>	77 (18.50)	30 (22.22)	33 (19.52)	14 (12.50)	0.1332	65 (31.10)	12 (5.79)	0.0000*	30 (15.78)	47 (20.79)	0.19	
Hookworm	04 (0.96)	03 (2.22)	01 (0.59)	00 (0.00)	/	02 (0.95)	02 (0.96)	/	01 (0.52)	03 (1.32)	/	
<i>H. nana</i>	01 (0.24)	00 (0.00)	01 (0.59)	00 (0.00)	/	00 (0.00)	01 (0.48)	/	00 (0.00)	01 (0.44)	/	

n : number of positive ; % : Frequency ;

P : P-value ; * : Statistically significant at P-value less than 0.05;

P: *Plasmodium*;

M: *Mansonella*;

L: *Loa*; E: *Entamoeba*;

G: *Giardia*;

A: *Ascaris*;

T: *Trichuris*;

H: *Hymenolepis*;

Em: *Embadomona*; En: *Endolimax*

3.3 Parasitic Infra-Communities

Amongst 416 schoolchildren recruited, only 124 (29.80%) were infected with only one parasite species and up to 185 (44.47%) were infected with two and more parasites species. The maximum number of parasite species found in a host was 5 and the mean specific richness was 1.43 ± 0.01 per individual. One hundred and eleven (26.68%) harbored two parasites species concurrently (bi-parasitism). There were 47 (11.29%) cases of 3 parasite infra-community (tri-parasitism); 24 (5.76%) cases of 4 parasites infra-community (quadri-parasitism); and 3 (0.72%) cases of 5 parasites infra-community (penta-parasitism). The Table 3 displays different parasitic infra-communities found in the study population.

Table 3. Different parasitic infra-communities observed according to the living area.

Type of infra-community	Parasite species	Rural		Urban		General	
		n	%	n	%	n	%
Biparasitism	<i>A. lumbricoides</i> + <i>E. coli</i>	07	1.68	03	0.72	10	2.40
	<i>A. lumbricoides</i> + <i>E. histolytica/dispar</i>	05	1.20	01	0.24	06	1.44
	<i>A. lumbricoides</i> + <i>G. intestinalis</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>M. perstans</i>	01	0.24	01	0.24	02	0.48
	<i>A. lumbricoides</i> + <i>P. falciparum</i>	05	1.20	03	0.72	08	1.92
	<i>A. lumbricoides</i> + <i>T. trichiura</i>	07	1.68	02	0.48	09	2.16
	<i>Blastocystis</i> sp + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>E. coli</i> + <i>E. histolytica/dispar</i>	11	2.64	5	1.20	16	3.84
	<i>E. coli</i> + <i>E. nana</i>	02	0.48	00	0.00	02	0.48
	<i>E. coli</i> + <i>G. intestinalis</i>	06	1.44	02	0.48	08	1.92
	<i>E. coli</i> + <i>P. falciparum</i>	05	1.20	05	1.20	10	2.40
	<i>E. coli</i> + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>E. histolytica/dispar</i> + <i>P. falciparum</i>	04	0.96	16	3.84	20	4.80
	<i>E. nana</i> + <i>P. falciparum</i>	00	0.00	02	0.48	02	0.48
	<i>G. intestinalis</i> + <i>P. falciparum</i>	01	0.24	01	0.24	02	0.48
	<i>M. perstans</i> + <i>P. falciparum</i>	00	0.00	01	0.24	01	0.24
	Hookworm + <i>P. falciparum</i>	00	0.00	01	0.24	01	0.24
	<i>T. trichiura</i> + <i>L. loa</i>	01	0.24	00	0.00	01	0.24
	<i>T. trichiura</i> + <i>P. falciparum</i>	07	1.68	01	0.24	08	1.92
	Total 1		66	15.86	45	10.81	111
Triparasitism	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. nana</i>	01	0.24	01	0.24	02	0.48
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>M. perstans</i>	03	0.72	00	0.00	03	0.72
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>T. trichiura</i>	05	1.20	03	0.72	08	1.92
	<i>A. lumbricoides</i> + <i>E. histolytica/dispar</i> + <i>P. falciparum</i>	02	0.48	00	0.00	02	0.48
	<i>A. lumbricoides</i> + Hookworm + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + Hookworm + <i>M. perstans</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	01	0.24	01	0.24	02	0.48

	<i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>E. nana</i>	01	0.24	01	0.24	02	0.48
	<i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>G. intestinalis</i>	01	0.24	00	0.00	01	0.24
	<i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>P. falciparum</i>	05	1.20	04	0.96	09	2.16
	<i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>T. trichiura</i>	04	0.96	00	0.00	04	0.96
	<i>E. coli</i> + <i>P. falciparum</i> + hookworm	00	0.00	01	0.24	01	0.24
	<i>E. coli</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	05	1.20	00	0.00	05	1.20
	<i>E. coli</i> + <i>M. perstans</i> + <i>T. trichiura</i>	00	0.00	01	0.24	01	0.24
	<i>E. histolytica/dispar</i> + <i>T. trichiura</i> + <i>G. intestinalis</i>	01	0.24	00	0.00	01	0.24
	<i>E. histolytica/dispar</i> + <i>T. trichiura</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>G. intestinalis</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	02	0.48	00	0.00	02	0.48
Total 2		35	8.41	12	2.88	47	11.29
	<i>A. lumbricoides</i> + <i>Blastocystis</i> sp. + <i>E. coli</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>Blastocystis</i> sp. + <i>P. falciparum</i> + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>P. falciparum</i>	02	0.48	00	0.00	02	0.48
Quadriparasitism	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>T. trichiura</i>	03	0.72	00	0.00	03	0.72
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. nana</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. nana</i> + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>M. perstans</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>M. perstans</i> + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	06	1.44	00	0.00	06	1.44
	<i>A. lumbricoides</i> + <i>E. histolytica/dispar</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	02	0.48	00	0.00	02	0.48
	<i>A. lumbricoides</i> + <i>G. intestinalis</i> + <i>M. perstans</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>Blastocystis</i> sp. + <i>E. coli</i> + <i>E. histolytica/dispar</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>E. coli</i> + <i>E. histolytica/dispar</i> + Hookworm + <i>M. perstans</i>	01	0.24	00	0.00	01	0.24
	<i>E. coli</i> + <i>G. intestinalis</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	02	0.48	00	0.00	02	0.48
Total 3		24	5.76	00	0.00	24	5.76
	<i>A. lumbricoides</i> + <i>E. coli</i> + <i>E. histolytica</i> / <i>dispar</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24

Pentaparasitism	<i>falciparum</i> + <i>T. trichiura</i>	01	0.24	00	0.00	01	0.24
	<i>A.lumbricoides</i> + <i>E. coli</i> + <i>E. nana</i> + <i>M. perstans</i> + <i>P. falciparum</i>	01	0.24	00	0.00	01	0.24
	<i>Blastocystis sp.</i> + <i>E. coli</i> + <i>E. intestinalis</i> + <i>P. falciparum</i> + <i>T. trichiura</i>	03	0.72	00	0.00	03	0.72
Total 4		03	0.72	00	0.00	03	0.72

n : number of cases ; % : Frequency .

3.4 Risks Factors of Monoparasitism and Multiparasitism

Table 4 displays the results of the logistic regression analysis which identifies associated factors of monoparasitism and multiparasitism. Age group and living area were associated with a high risk of monoparasitism. In fact, schoolchildren aged from 8 to 11 years were almost twice as likely to be infected with single parasite compared to those aged from 4 to 7 years (aOR = 1.92 ; 95% CI: 1.03 - 3.59 ; $P = 0.0403$). Schoolchildren living in urban areas were less likely to be infected with one parasite than those living in rural areas (aOR = 0.56; 95% CI: 0.32 - 0.99; $P = 0.0492$). The risk of multiparasitism was significantly influenced by gender, age group and living areas. In fact, the risk was higher for females (aOR = 2.12; 95% CI: 1.26 - 3.57; $P = 0.0046$) and schoolchildren aged from 8 to 11 years (aOR = 1.70; 95% CI: 1.09 - 3.15; $P = 0.0089$) compared to males and those aged from 4 to 7 years, respectively. In contrast, the risk of multiparasitism was low among schoolchildren living in urban areas (aOR = 0.16; 95%CI: 0.09 - 0.28; $P = 0.0001$) compared to those living in rural areas.

Table 4. Associated variables of risk of Monoparasitism and multiple Multiparasitism

Variables	Monoparasitism				Multiparasitism			
	Crude OR [95%CI]	P	Adjusted OR [95%CI]	P	Crude OR [95%CI]	P	Adjusted OR [95%CI]	P
Gender								
Male	1		1		1		1	
Female	1.23 [0.73-2.06]	0.4382	1.26 [0.74 -2.13]	0.3904	1.99 [1.22 -3.22]	0.0055*	2.12 [1.26 - 3.57]	0.0046*
Age Group								
4 - 7 ans	1		1		1		1	
8 - 11 ans	2.03 [1.10-3.77]	0.0243*	1.92 [1.03 - 3.59]	0.0403*	1.98 [1.12 - 3.50]	0.0192*	1.70 [1.09 - 3.15]	0.0089*
12 - 15 ans	1.20 [0.62-2.32]	0.5895	1.35 [0.69 - 2.65]	0.3805	1.24 [0.68 - 2.25]	0.4901	1.91 [1.02 - 3.71]	0.0551
Living area								
Rural	1		1		1		1	
Urban	0.53 [0.30 -0.92]	0.0246*	0.56 [0.32 - 0.99]	0.0492*	0.17 [0.10 - 0.28]	<0.0001*	0.16 [0.09 - 0.28]	<0.0001*

OR: Odd Ratio; 95%CI: 95% Confidence Interval; P: P-value; (*): Significant

3.5 Parasitic Associations

All significant association ($P < 0.05$) between parasites, gender, age groups and living areas resulting from multivariable logistic regression are summarized in Table 5. *Trichuris trichiura* showed a significant and positive association with *Ascaris lumbricoides* (aOR = 2.49; 95%CI = 1.39 - 4.43) and *Entamoeba coli* (aOR = 2.95; 95%CI = 1.70 - 5.13) but a significant and negative association with the urban area. A positive association was found between *Mansonella perstans* and *Ascaris lumbricoides* (aOR = 1.79; 95% CI = 1.06 - 5.09) but a negative association with the urban setting (aOR = 0.32; 95% CI = 0.11 - 0.94). *Entamoeba histolytica/dispar* showed a positive association with *Trichuris trichiura* (aOR =

2.10; 95% CI = 1.04 - 4.20), *E. coli* (aOR = 4.35; 95%CI = 2.61 - 7.25) and the age group between 8 and 11 years (aOR = 2.06; 95%CI = 1.11 - 3.85). A significant and negative association was found between *Plasmodium falciparum* and the urban environment (aOR = 0.62; 95%CI = 0.41 - 0.93).

Table 5. Association between a particular parasitic and gender, age group and any remaining parasites

Parasites	Co-variables	aOR (95%CI)	P
<i>T. trichiura</i>	<i>A. lumbricoides</i>	2.49 (1.39-4.43)	<0.001
	<i>E. coli</i>	2.95 (1.70-5.13)	<0.001
	Rural area	0.14 (0.07 – 0.26)	< 0.001
<i>Lumbricoides</i>	<i>E. coli</i>	4.95 (2.87 – 8.48)	0.011
	<i>M. perstans</i>	1.79 (1.06 – 5.09)	0.001
	Female	1.71 (1.03 – 2.85)	0.04
	Rural area	0.19 (0.10 – 0.33)	< 0.0001
<i>M. perstans</i>	Rural area	0.32 (0.11 – 0.94)	0.04
<i>E. histolytica/dispar</i>	<i>T. trichiura</i>	2.10 (1.04 – 4.20)	0.036
	<i>E. coli</i>	4.35 (2.61 – 7.25)	0.001
	[8 -11[years	2.06 (1.11 – 3.85)	0.02
<i>E. coli</i>	Rural area	0.24 (0.15 – 0.40)	< 0.001
<i>P. falciparum</i>	Rural area	0.62 (0.41 – 0.93)	0.02

Adjusted OR: Odd Ratio; 95%CI: 95% Confidence Interval; P: P-value.

4. Discussion

The study showed that the frequency of multiparasitism is higher than that of monoparasitism in Akonolinga, Nyong et Mfoumou Division, Centre Region of Cameroon. Approximately $\frac{3}{4}$ of the population studied were infected with at least one parasitic species. This infection rate is lower than that found by Kimbi *et al.* (2012) more than 82%, among schoolchildren in the South-West Region of Cameroon. It is similar to that of Zeukeng *et al.* (2014) 77.2% among general population in the Centre Region of the same country. Conversely, our finding is higher than 26.6% obtained by Lehman *et al.* (2012), and 8.5% by Khan Payne *et al.* (2017) in Littoral, Centre and West regions of Cameroon respectively. This shows that despite the fact that schoolchildren are the main target of parasitic infection control strategies, they are still the segment of the population most vulnerable to parasitic infections. The highest prevalence was found in schoolchildren of rural areas. As we noticed in the rural area, drinking unsafe water, wearing open shoes and using latrines, if they existed, inappropriately maintained by children could justify these results.

A total of 13 parasitic species were found in this study population. Ten of them were also reported by M'bondoukwe *et al.* (2013) in Gabon (*Plasmodium falciparum*, *Loa loa*, *Mansonella perstans*, *Giardia intestinalis*, *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Blastocystis sp.*, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm). Similarly, Raso *et*

al. (2004) and Coulibaly *et al.* (2012) reported both in Côte d'Ivoire, nine and eight parasite species found in our study (Hookworm, *Trichuris trichiura*, *Ascaris lumbricoides*, *Entamoeba coli*, *Entamoeba histolytica/dispar*, *Blastocystis sp.*, *Endolimax nana*, *Giardia intestinalis* and *Plasmodium falciparum*). In the same country were found 8 species of intestinal parasites out of the 10 obtained in our study. These results confirm that in sub-Saharan Africa, environmental and climatic conditions are favorable for the development and persistence of several parasite species.

Plasmodium falciparum being the only species of that genus found and this study confirms that it is the main malarial agent in Cameroon as previously reported by Kimbi *et al.* (2012) and Khan Payne *et al.* (2017). Malaria prevalence was higher in the rural area; this is consistent with findings of Olurongbe *et al.* (2011) and Kimbi *et al.* (2012) and may be due to higher risk of contact with mosquito vectors as a result of a higher presence of their breeding sites, lower level of education and lower rate of preventive methods against malaria in this area compared with urban ones (Kimbi *et al.*, 2012).

Entamoeba coli (29.33%) and *Entamoeba histolytica/dispar* (23.80%) were the commonest intestinal protozoa found in this study. The prevalence of the former protozoa is similar to that reported by Coulibaly *et al.* (2012) in Côte d'Ivoire (31.8%) but higher than that obtained by M'bondoukwé *et al.* (2018) in Gabon (22.2%). As regards *Entamoeba histolytica/dispar*, its overall prevalence was higher than those obtained by the above mentioned authors who had values of 7.4 % and 9.3% respectively. The higher prevalence of amoebae in this study could be justified by the fact that study period coincided with the harvest and increased consumption of fruits such as mangoes. It should be noticed that a large proportion of children, especially in rural areas, were consuming fruits without prior washing them and/or washing their hands.

Ascaris lumbricoides (21.4%) and *Trichuris trichiura* (18.5%) were the main parasitic helminths found in this study. High prevalence of *Ascaris lumbricoides* and *Trichuris trichiura* observed among intestinal helminths could be explained by the fact that both are fecal-orally transmitted and their epidemiology dependent on individual and community hygienic habits and human waste disposal methods and then, subsequently affect the level of environmental contamination. Ova from both species are equipped with outer coat that enables them to resist adverse external environmental conditions and enhances their survival and higher probability of transmission (Ruto & Mulambalah, 2016). Besides, the prevalence of both soil transmitted helminths were higher than those reported by Khan Payne *et al.* (2017) in the West Region of Cameroon (*Ascaris lumbricoides* 4% and *Trichuris trichiura* 4.1%) and M'Bondoukwé *et al.* (2018) in Gabon (*Ascaris lumbricoides* 13.7% and *Trichuris trichiura* 11.8%). However, these findings are lower than those found by Kimbi *et al.* (2012) in the South West Region of Cameroon (*Ascaris lumbricoides* 30.21% and *Trichuris trichiura* 25.98%) and Ruto and Mulambalah (2016) in Kenya (*Ascaris lumbricoides* 55.8% and *Trichuris trichiura* 26.9%).

Approximately 45% of the study population was infected with two or more parasites. The maximum number of parasitic taxa found in a simple host was 5 and the mean specific

richness was 1.43 ± 0.01 parasites per individual. Our finding agrees with Tchuem Tchuente *et al.* (2004) and Kimbi *et al.* (2012) in Cameroon; Raso *et al.* (2004) and Hürlimann *et al.* (2014) in Côte d'Ivoire; Ruto and Mulambalah (2016) in Kenya. These findings are in line with the statement of Petney and Andrews (1988) on the fact that: “multiparasitism is the rule rather than the exception in most biological systems and the co-infection rate can reach 80% in some human populations”. The biparasitism was the main parasitic association in this study with the association *Plasmodium falciparum* + *Entamoeba histolytica/dispar* primarily reported (18%) among children. This can be attributed to the high prevalence rates and local endemic of these parasites in the area.

Statistically significant associations were found between some parasitic taxa especially between *Ascaris lumbricoides* and *Trichuris trichiura* and between *Entamoeba coli* and *Entamoeba histolytica/dispar*. Such observations were also previously reported by Raso *et al.* (2004), Coulibaly *et al.* (2012) and Hürlimann *et al.* (2014). These parasitic taxa share the same routes of transmission to humans through ingestion of contaminated food or drinking water with parasite infesting development stages. The lack of hygiene and poor health conditions can also favor the transmission of these parasites. The significant association between *Ascaris lumbricoides* and *Mansonella perstans*, two helminths with different transmission mode (oral route for *Ascaris lumbricoides* and bite of midge arthropod for *Mansonella perstans*) and ecological niches in the host (Intestine for *Ascaris lumbricoides* and blood for *Mansonella perstans*), would mean a synergy between these parasites via host immunity. Indeed, all helminthiasis are chronic infections hallmarked by a strong immune response to Th2 cell-mediated dominated by an increase in anti-inflammatory cytokines (Bwanika *et al.*, 2018). Thus, the infection with a helminth specie would create conditions favorable to the infections of other helminths specie within the host.

5. Conclusions

Our study pointed out that parasitic infections are highly prevalent in Akonolinga especially *Plasmodium falciparum*, *Ascaris lumbricoides*, *Entamoeba coli* and *Entamoeba histolytica/dispar* infections. The frequency of multiparasitism is higher than that of monoparasitism in Akonolinga, Nyong et Mfoumou Division. Among parasitic infra-communities found, bi-parasitism was more frequent. The study also outlined many parasitic statistical associations. These findings should be helpful in defining and implementing more strong and effective parasitic disease control strategies in the Nyong et Mfoumou Division.

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Conflict of Interest

There is no conflict of interest between the authors of this publication.

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