Original Research Article

Co-occurrence of intestinal parasites among school children of Akonolinga, Centre Region of Cameroon: emergency need to reduce the health divide

ABSTRACT

Background: Intestinal parasitoses pose a serious problem to public health and development, especially for the underprivileged population of low and middle-income countries. School children, who are the most affected, can harbour several parasites at the same time. There is a need to adopt efficient strategies for the elimination of intestinal parasitic infections as a public health problem by 2030.

Methods: This cross-sectional study was conducted to highlight the level of co-occurrence of intestinal parasites among pupils of the Akonolinga locality of the Centre region of Cameroon. Pupils were recruited from 5 public schools; stool samples were collected from those whose parents agreed to participate in the study. Helminth~~s~~ eggs and protozoan cysts were assessed by direct smear and the formol-ether concentration technique.

Results: Out of the 416 pupils recruited, 252 were infected by at least one of the ten intestinal parasites identified. The most frequent soil-transmitted helminths were *Ascaris lumbricoides* and *Trichuris trichiura* with prevalences of 21.4 % and 18.5 %, respectively. *Entamoeba coli* was the main protozoan followed by *E. histolytica/dispar* with prevalences of 29.3% and 23.8%, respectively, and *Embadomonas intestinalis* was found in only one pupil (0.2%). The co-occurrence of intestinal parasites reached 50% and children with up to four different parasites were detected. The pair of intestinal protozoan parasites, *E. histolytica/dispar* and *E. coli* ~~was~~ were the most observed with a frequency of 9.5%. Living in a rural setting and young age were important determinants for most of the parasite infections and co-occurrence of parasites.

Conclusion: The co-occurrence of intestinal parasites among school children of Akonolinga is high, and this situation is more alarming in ~~the~~ rural areas and in younger children. The living conditions and the co-occurrence of parasites must be integrated ~~in~~ into public policies ~~of~~ for fighting against intestinal parasite infections.

Keywords: Intestinal parasites; Co-occurrence; School children; Health divide; Akonolinga; Rural area.

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1. INTRODUCTION

Ecological communities include a complex network of biotic interactions such as predation, mutualism, competition, facilitation and parasitism [1]. For parasites, hosts can be considered as ecosystems that have easily defined resources [2]. In most cases, the host is infected simultaneously by several parasite species; as a result of common ecological and environmental requirements, infection routes, host exposure, host susceptibility, behavioural, sociological, and economic factors that enable the co-occurrence of parasite-host systems [3, 4]. Co-infections have important public health, epidemiological and evolutionary implications. They can lead to different outcomes for both the host and the parasites compared to single parasite infection. For instance, co-infections can increase the parasite virulence evolution rate and lead to higher morbidity and mortality of the hosts. The within-host interactions between parasites can regulate their coexistence with mutually beneficial, antagonistic or neutral impacts on one another, leading to higher or lower reproductivity [5, 6].

Intestinal helminths and protozoa are among the most important causes of infections in developing countries, especially in tropical and subtropical countries carrying high burdens of morbidity and mortality [7]. Epidemiological evidence suggests that the soil-transmitted helminths, *Ascaris lumbricoides, Trichuris trichiura* and Hookworms, affect more than 1.5 billion people; more than 267 million preschool children and 568 million school children live in areas where these parasites are intensively transmitted [8]. Regarding protozoa species, *Cryptosporidium* spp., *Entamoeba histolytica* and *Giardia intestinalis* are the most common protozoans infecting children in sub-Saharan Africa [9]. School-aged children are the most affected, due to their habits of playing or handling infected soil, eating with soiled hands, unhygienic toilet practices, drinking and eating contaminated water and food respectively [10]. Intestinal parasites consume nutrients from the children they infect, thus retarding their physical and cognitive development. They destroy tissues and organs, cause abdominal pain, diarrhoea, intestinal obstruction, anaemia, ulcers, and other health problems which can lead to slow cognitive development and impaired learning [10, 11].

In 2015, the United Nations adopted 17 Sustainable Developments Goals (SDGs) to end poverty, protect the planet, and ensure that by 2030 all people enjoy peace and prosperity [12]. In order to attain the SDG 3: « Ensure healthy life and promote well-being for all at all ages», WHO proposed a road map for NTDs for 2021–2030 [13]; so, there is a need to adopt efficient strategies. Hence, research on the co-occurrence of parasites, increasingly recognized as the norm, could be a prerequisite to designing effective strategies to guarantee the elimination of intestinal parasitic infections as a public health problem. In Cameroon, intestinal parasites are distributed all over the country and several studies have been conducted to prove this [3, 14 - 16]. However, there are still several localities for which epidemiological information is needed. In this paper we examined the co-occurrence and health impact of intestinal parasites among school children of the Akonolinga sub-division, Centre Region of Cameroon.

2. MATERIAL AND METHODS

2.1. Study Area

This study was carried out between September 2017 and July 2018 in the locality of Akonolinga in the centre region of Cameroon. Akonolinga is located 100 km east ~~from~~ of Yaoundé (the political capital of Cameroon). It is the Head Quarters of the Nyong and Mfoumou Divison, and situated at 3°46' North latitude, 12°15' East longitude, and 673 meters a.s.l. It covers an area of about 1,420 Km^2 with approximately 18.000 inhabitants [17]. The environment is predominantly tropical forest and the division is irrigated by the Nyong River system which flows from east to west.

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2.2. Study population

The study population consisted of school children of the Akonolinga locality. Four hundred and sixteen (416) pupils aged 4 to 15 years old were sampled in five primary schools: three in a rural area (Essang-Ndibi, Kpwele, Eboa) and two in an urban area (Loum, centre ville).

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2.3. Study Design

This study was approved by the National Ethical Committee of Research for Human Health (Ethical Clearance N°: 2018/01/968/CE/CNERSH/SP) and the Directorate of the Yaoundé University Teaching Hospital (Research Authorization N°: 894/AR/CHUY/DG/DGA/DMT). In this cross-sectional study; before samples were collected, informed consent was obtained from the parents and/or legal guardians of the pupils after they understood the purpose of the study. A questionnaire was used for the collection of socio-demographic and behavioural data. Each child received information on how to collect stool samples in order to avoid contamination with urine or other contaminants. Early-morning stool samples were collected in plastic screw-cap vials, labelled with each participant's information. All stool samples collected were put in an ice bag and transported to the Parasitology laboratory at the Yaoundé University Teaching Hospital, for parasitological analysis. Children whose parents or guardians did not give their consent, those who were recently enrolled in school, and those who had taken anti-helminthic treatment at least one month before, were excluded from the study as well as participants who didn't provide stool samples.

2.4. Parasitological examination and Morphological identification

Stool samples were analysed using direct smears in saline and Lugol solution, and also by the formalin-ether concentration technique as described by Allen and Ridley [18]. Briefly, about a pea size of stool samples were emulsified in 7 ml of formalin, sieved through a wire gauze into an evaporating basin, and then transferred into a centrifuge tube. Then, 3 ml of ether were added, the mixture agitated vigorously for 30 seconds and centrifuged at 3,000 rpm for 60 seconds. The layer of debris accumulated at the interphase was removed by passing a swab stick gently around the circumference of the tube. The supernatant was discarded and the pellet was homogenised and mounted on a glass slide and cover slip for microscopic examination. Samples were observed at a magnification of x200 and x400 using the CyScope® microscope (Partec-Sysmex GmbH, Görlitz, Germany) and the identification of parasite eggs or cysts was done using photos and dimension ranges according to the WHO Bench Aid for the diagnosis of intestinal parasites. All stool samples were analysed in duplicate.

2.5. Statistical Analysis

Data obtained were entered and stored in a Microsoft Excel spread sheet then exported to the SPSS (Statistical Package for Social Sciences) version 25.0 software for subsequent analyses. Prevalence was defined as the number of individuals infected with a particular parasite species x 100, divided by the total number of examined individuals [19], while the co-occurrence rate was defined as the number of simultaneous appearances of different parasite species x 100, divided by the total number of infected individuals. The prevalences of different intestinal parasites were compared between age groups, genders, and living areas, using the chi-square test (χ^2). The logistic regression model was performed to determine the risk factor(s) of co-occurrence of intestinal parasite infections. Prevalence and co-occurrence rates were tested at 5% significance level ($P < 0.05$).

3. RESULTS

A total of 416 stool samples were collected from school children and examined during the current study. One hundred and sixty-four (39.4%) children were uninfected while 252 (60.6 %) were infected with at least one intestinal parasite (protozoa and/or helminth). Intestinal protozoans were present in 199 (47.8%) cases, and helminths in 135 (32.5%) cases.

Amongst the Ten (10) parasite species recorded, four (04) were helminths and six (06) were protozoans. Helminth species included *Ascaris lumbricoides* Linnaeus, 1758, *Trichuris trichiura* Linnaeus, 1771, *Hymenolepis nana* Siebold, 1852, and Hookworms (*Necator americanus* Stiles, 1902 / *Ancylostoma duodenale*, Dubini, 1843). Protozoan species included *Entamoeba histolytica/dispar* Schaudinn 1903/Brumpt, 1925, *E. coli* Loesch, 1875, *Endolimax nana* Kuenen et Swellengrebel, 1917, *Giardia intestinalis* Kofoid et Christiansen, 1915, *Embadomonas intestinalis* Grassi, 1879 and *Blastocystis* sp. Alexieff, 1911. The most prevalent species among helminths and protozoa were *A. lumbricoides* (21.4%) and *E. coli* (29.3%) respectively as ~~showed~~ shown in Figure 1 below.

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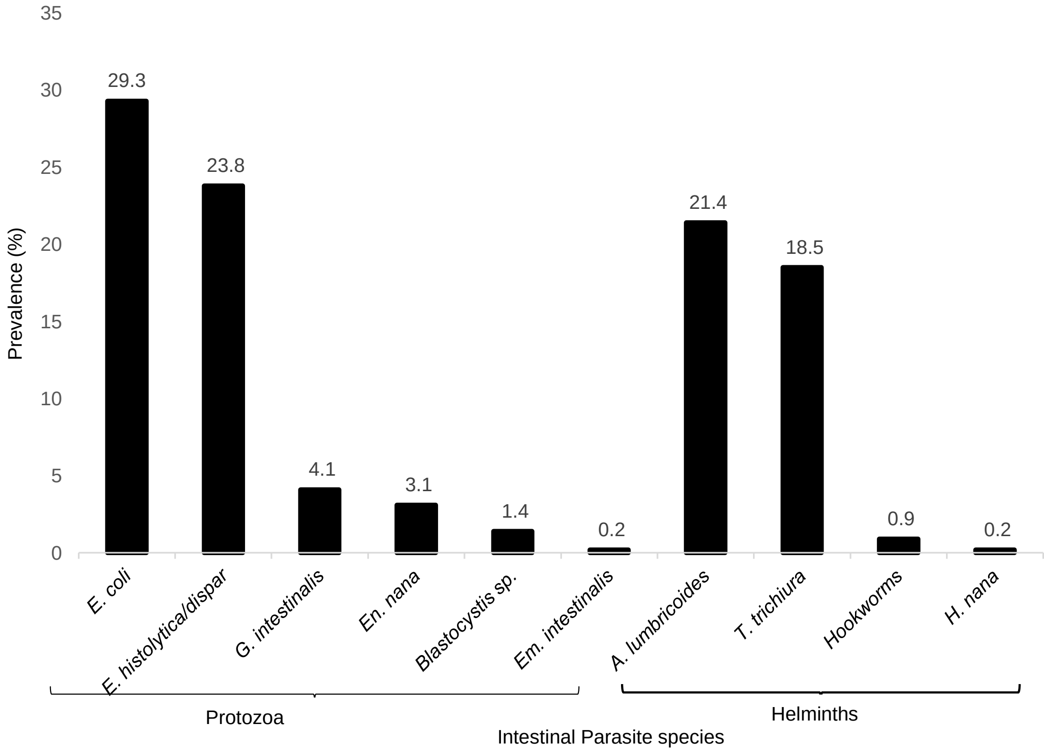

Figure 1: Prevalence of intestinal parasites in pupils of the Akonolinga locality.

The prevalence ~~or~~ of infection rates of intestinal parasites according to living areas, age groups and gender are presented in table 1. The prevalence of *E. coli, G. intestinalis*, *A. lumbricoides* and *T. trichiura* were significantly higher in the rural areas compared to the urban areas. Although not significant (P> 0.05), the prevalence of *E. coli*, *Blastocystis* sp., *Em. intestinalis*, *En. nana*, *T. trichiura* and Hookworms, were higher in children aged 4 to 8 years old. Conversely, the prevalence of *E. histolytica/dispar* (P = 0.042) and *A. lumbricoides* (P = 0.255) tended to increase significantly with age. In most cases (except *Em. intestinalis* and *En. nana*), females were more often infected than males.

Table 1: Prevalence of intestinal parasites according to living areas, age groups and gender

Parasite species	**Living area**			**Age group**				**Gender**		
	Rural (N_1 = 209)	**Urban (N_2 = 207)**	***P***	**[4 – 8[(x_1 = 135)**	**[8 – 12[(x_2 = 169)**	**[12 – 16 [(x_3 = 112)**	***P***	**Female (n_1 = 226)**	**Male (n_2 = 190)**	***P***
E. coli	89 (42.6)	33 (16.0)	0.001*	42 (31.1)	51 (30.2)	29 (25.9)	0.636	74 (32.7)	48 (25.3)	0.095
E.histolytica/dispar	46 (22)	53 (25.6)	0.389	22 (16.3)	45 (26.6) $	32 (28.6) $	0.042*	61 (27.0)	38 (20.0)	0.095
G. intestinalis	13 (6.2)	04 (1.9)	0.027*	04 (2.9)	10 (5.9)	03 (2.7)	0.294	04 (1.7)	13 (6.8) $	0.027*
Blastocystis sp.	06 (2.8)	00 (0.0)	/	03 (2.2)	01 (0.6)	02 (1.4)	0.465	04 (1.7)	02 (1.1)	0.541
Em. intestinalis	01 (0.5)	00 (0.0)	/	01 (0.7)	-	-	/	-	01 (0.5)	/
En. nana	08 (3.9)	05 (2.4)	0.407	06 (4.4)	04 (2.4)	03 (2.7)	0.556	06 (2.6)	07 (3.7)	0.547
A.lumbricoides	70 (33.5)	19 (9.2) $	0.000*	23 (17.0)	42 (24.8)	24 (21.4)	0.255	57 (25.2) $	32 (16.8)	0.039*
T. trichiura	65 (31.1)	12 (5.8) $	0.000*	30 (22.2)	33 (19.5)	14 (12.5)	0.133	47 (20.8)	30 (15.8)	0.19
Hookwoms	02 (0.9)	02 (0.9)	/	03 (2.2)	01 (0.6)	00 (0.0)	/	03 (1.3)	01(0.5)	/
H. nana	00 (0.0)	01 (0.5)	/	-	01 (0.5)	-	/	01 (0.5)	00 (0,0)	/

N_1: number of schoolchildren recruited in rural area; **N_2**: number of schoolchildren recruited in urban area; **x_1:** number of schoolchildren aged 4 to 7 years; **x_2**: number of schoolchildren aged 8 to 11 years; **x_3:** number of schoolchildren aged 12 to 15 years; **n_1**: number of female in the study population; **n_2**: number of male in the study population; **E:** *Entamoeba*; **G:** *Giardia*; **Em**: *Embadomonas*; **En**: *Endolimax*; **A**: *Ascaris*; **T**: *Trichiura*; **H**: *Hymenolepis;* *: P-value significant; **$**: Prevalence significantly high.

Co-occurrence of parasite species was recorded in 126 (50%) of the 252 pupils found infected by at least one parasite species. The majority of simultaneous infections consisted of two parasite species (33.7%, 85/252), then three parasite species (13.1 %; 33/252), and only 3.2% (8/252) cases with four parasite species. Figure 2 below illustrates the pattern of the different types of parasitic co-occurrence. The most prevalent (co-occurrence > 5%) parasitic co-infections were in decreasing order E. *histolytica/dispar* + *E. coli* (9.5 %; 24/252), *A. lumbricoides* + *E. coli* (6.3 %; 16/252), *T. trichiura* + *E. coli* (6.3 %; 16/252) and *A. lumbricoides* + *T. trichiura* + *E. coli* (6.0 %; 15/252). Apart from *H. nana*, all other parasite species were involved in co-infections; *A. lumbricoides* (87.3 %; 110/126) was the most involved in parasite co-infections, followed by *E. coli* (71.4 %, 90/126) and *T. trichiura* (47.6 %; 60/126).

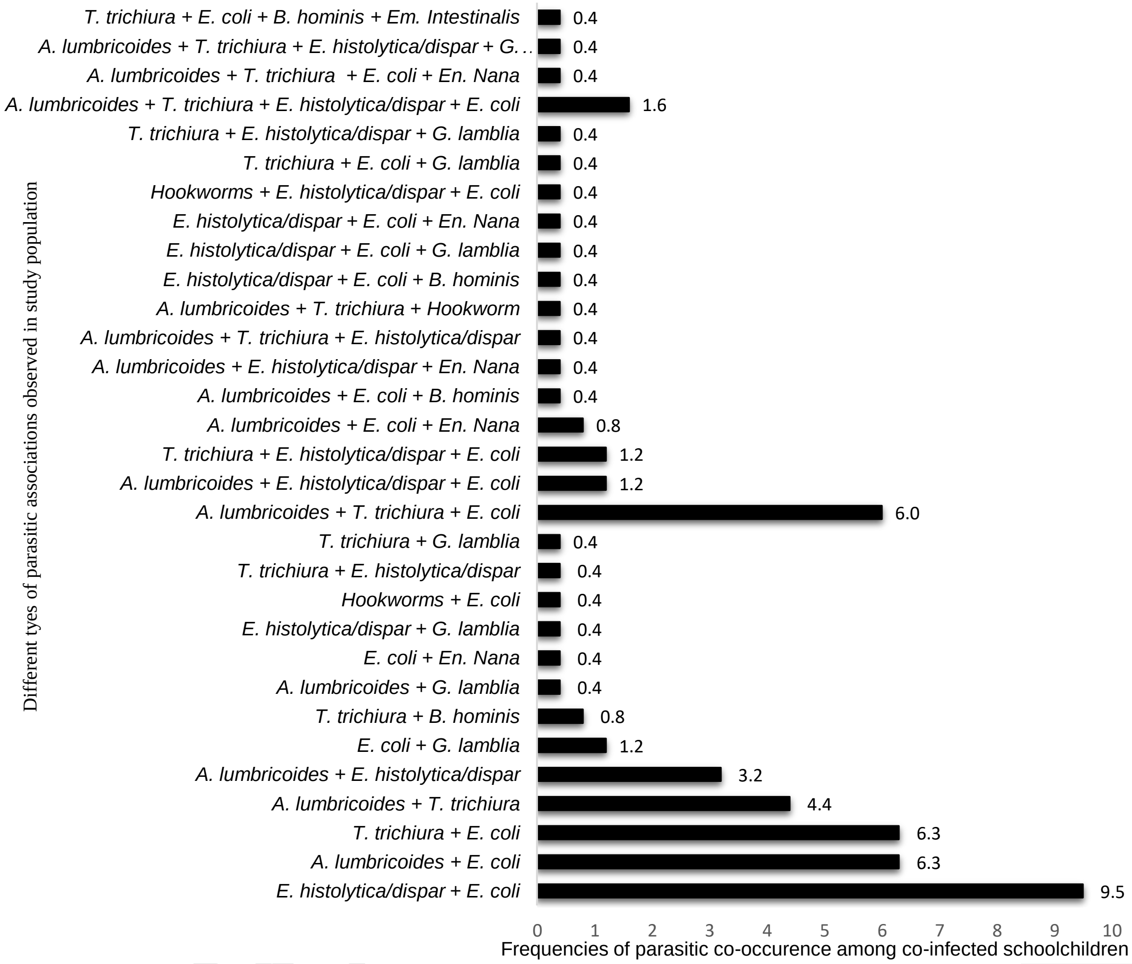

Figure 2: Patterns of different types of parasitic co-occurrences in school children of Akonolinga.

Table 2 below outlines some of the risk factors involved in single infections and co-occurrent intestinal parasitic infections. Only single and concomitant parasitic infections with frequency ≥ 5 % were included in the model of logistic regression. Rural areas appeared as the main risk factor for both single and co-occurrent intestinal parasitic infections. This was the case with single infections by: *A. lumbricoides* (OR = 1.80; 95% CI:1.10 – 3.30; *P*< 0.001), *T. Trichiura* (OR = 1.30, 95%CI: 1.07 – 2.60, *P*< 0.001), and *E. coli* (OR = 2.40, 95%CI: 1.50 – 4.00, *P*< 0.001), and with concomitant infections by: *T. trichiura* + *E. coli* (OR = 1.16 , 95%CI: 1.60 – 4.20, *P*< 0.001), *E. histolytica/dispar* + *E. coli* (OR = 2.60, 95% CI: 1.20 – 5.80, *P*< 0.001), *A. lumbricoides* + *E. coli* (OR = 1.70, 95%CI: 1.17 – 4.20, *P*< 0.001) and *A. lumbricoides* + *T. Trichiura* + *E. coli* (OR = 1.13 , 95%CI: 1.30 – 4.70, *P*< 0.001). The 4 - 7 years old age group was significantly (*P*<0.05) associated with *E. histolytica/dispar* infection.

Table 2: Risk factors associated with single infection and co-occurrent intestinal parasitic infections in school children of the locality of Akonolinga

Variable		**S.E.**	**Khi Square**	**P-value**	**OR**	**95 % CI**
A. lumbricoides	Female	0.26	3.29	0.07	0.62	0.37 – 1.04
	Age		3.68	0.16		
	[4 – 7] years	0.35	3.66	0.06	0.51	0.26 – 1.02
	[8 – 11] years	0.32	1.02	0.31	0.72	0.38 – 1.36
	Rural	0.30	32.6	0.000*****	**1.80**	**1.10 – 3.30**
T. trichiura	Female	0.27	1.13	0.28	0.75	0.43 – 1.27
	Age		2.01	0.36		
	[4 – 7] years	0.38	1.08	0.29	1.48	0.71 – 3.11
	[8 – 11] years	0.37	0.000	0.98	0.99	0.48 – 2.06
	Rural	0.34	34.6	0.000*****	**1.30**	**1.07 – 2.60**
E. histolytica	Female	0.24	3.34	0.06	0.64	0.41 - 1.03
	Age		6.58	0.03		
	[4 – 7] years	0.32	5.19	0.02*	**0.48**	**0.26 – 0.90**
	[8 – 11] years	0.28	0.03	0.84	0.94	0.54 – 1.65
	Rural	0.24	0.74	0.38	1.23	0.77 – 1.98
E. coli	Female	0.23	2.29	0.13	0.70	0.45 – 1.11
	Age		0.87	0.64		
	[4 – 7] years	0.30	0.001	0.96	0.98	0.54 – 1.79
	[8 – 11] years	0.29	0.58	0.44	0.79	0.45 -1.43
	Rural	0.24	33.0	0.000*****	**2.40**	**1.50 – 4.00**
T. trichiura + E. coli	Female	0.37	0.90	0.34	0.70	0.39 – 1.46
	Age		1.73	0.42		
	[4 – 7] years	0.48	0.87	0.34	0.64	0.25 – 1.63
	[8 – 11] years	0.46	1.68	0.19	0.55	0.23 – 1.35
	Rural	0.47	14.5	0.000*****	**1.60**	**1.16 – 4.20**
E. histolytica/dispar + E. coli	Female	0.35	0.70	0.40	0.74	0.37 – 1.48
	Age		0.10	0.94		
	[4 – 7] years	0.47	0.01	0.90	0.94	0.38 – 2.37
	[8 – 11] years	0.45	0.09	0.75	0.87	0.36 – 2.10
	Rural	0.40	10.7	0.001*****	**2.60**	**1.20 – 5.80**
A.lumbricoides + E. coli	Female	0.34	0.000	0.99	1.00	0.51 – 1.96
	Age		0.74	0.69		
	[4 – 7] years	0.47	0.74	0.38	0.66	0.26 – 1.68
	[8 – 11] years	0.43	0.26	0.60	0.80	0.34 – 1.87
	Rural	0.44	15.36	0.00*****	**1.70**	**1.17 – 4.20**
A.lumbricoides + T. Trichiura + E. coli	Female	0.47	1.95	0.16	1.94	0.76 – 4.96
	Age		2.81	2.45		
	[4 – 7] years	0.62	1.94	0.16	0.42	0.12 – 1.42
	[8 – 11] years	0.56	2.26	0.13	0.42	0.14 – 1.29
	Rural	0.65	9.64	0.000*****	**1.13**	**1.30– 4.70**

Legend**: SE**: Standard Error; **OR**: Odds Ratio; **E**: *Entamoeba*; **G**: *Giardia*; **Em**: *Embadomonas*; **En**: *Endolimax*; **A**: *Ascaris*; **T**: *Trichiura*; **H**: *Hymenolepis;* *****: P-value significant.

4. DISCUSSION

It is increasingly recognized that host co-infection with multiple pathogens is the norm and that a better understanding of the nature and extent of multi-parasitism can have important epidemiological, clinical and control implications [20]. The human intestinal tract harbours a community of micro-organisms including viruses, bacteria, fungi, protozoa and helminths [21]. Recent research has revealed interactions between micro-organisms in the gut, with effects on the parasites themselves as well as on host nutrition and organ pathology [22 - 24]. In the current work, we conducted a study with the aim of highlighting the extent of the co-occurrence of intestinal parasites among school children in the Akonolinga locality (Centre region of Cameroon).

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In low and middle-income countries, children bear the greatest burden of intestinal parasitic infections in terms of morbidity and mortality. In children, intestinal parasitic infections, especially helminthiasis, may cause a number of negative health outcomes such as malnutrition, anaemia, impaired growth and cognitive development. Additionally, these infections may contribute substantially to increase the burden of poverty, impaired mental and educational development in children and damage economic productivity [25 - 27].

Our results revealed the circulation of at least 10 intestinal parasite species in the locality of Akonolinga. Many of them, nematodes (*A. lumbricoides*, Hookworms and *T. trichiura*) and Amoebae (*E. histolytica/dispar* and *E. coli*), were found at different infection rates in many other localities in Cameroon: Littoral [28], West [15], South [29], and in the South-west [30] regions. The variation in the prevalence may be explained by the geographical and ecological factors, behavioural habits and living conditions of the study populations. High prevalences of intestinal parasitic infections are still common in school children as indicated by this current study which showed a high intestinal parasite infection prevalence rate of 60.6%. These results suggest that the de-worming program put in place may be insufficient or ineffective in eradicating the infection, particularly in this population. The prevalences of many parasites were significantly higher in the rural setting compared to the urban setting, especially for *A. lumbricoides*, *T. trichiura*, *E. coli* and *G. intestinalis*. In low and middle-income countries, rural communities face many difficulties such as poverty, poor sanitation, untreated water and inadequate drainage systems, poor healthcare, promiscuity and dwellings made up of temporary materials [31, 32]. All these features contribute to poor living conditions in rural areas, and thus a ~~wide spread~~ widespread and a stable transmission of several intestinal parasite species whose transmission stages have the capacity to resist for a long time in the environment [33]. These parasitic infections cause socio-economic devastation and hamper healthcare in rural vulnerable communities [34 - 36]. There is therefore an emergent need to reduce the health divide through the promotion of mass de-worming or parasite control, health education and access to ~~health care~~ healthcare facilities in rural areas. This is a prerequisite to the elimination of intestinal parasitic infections as a public health problem, towards the attainment of ~~the~~ sustainable health development goals.

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This study also revealed that co-occurrence of intestinal parasites occurs in one out of two cases (50%) and that a single individual can simultaneously harbour up to four parasite~~s~~ species. Our findings are similar to published data and confirm~~s~~ that in general, ~~the~~ multiparasitism is the rule rather than the exception [37 - 39]. Both single parasite and multiparasite infections had common risk factors mostly related to the children's individual conditions and habits. In fact, the simultaneous presence of many parasite species can lead to synergistic or antagonistic interspecific interactions between parasites. Such interactions can have important repercussions on the host's health because they can alter host's susceptibility to other parasites, infection duration, transmission risks, clinical symptoms and consequently treatment and prevention strategies [39].

5. CONCLUSION

In the Akonolinga locality, the co-occurrence of intestinal parasites among school children is quite frequent, mainly in the rural areas where the highest prevalence of many intestinal parasite infections was recorded. To ensure a healthy life and well-being for all, it is important to reduce the health divide through the promotion of mass deworming or parasite control, health education and access to healthcare facilities in rural areas. Furthermore, it's also important to develop new approaches that will help highlight the co-occurrence of parasites and integrate this neglected reality ~~in~~ into strategies for the elimination of intestinal parasitic infections as a public health problem by 2030.

6. STUDY LIMITATION

The fact that only a single stool sample was analysed per child, ~~which~~ may underestimate the prevalence of intestinal parasites and this could be a possible limitation ~~to~~ of this study. However, to minimize this situation, each sample was also examined by using the formalin-ether concentration technique; thus, increasing the diagnostic accuracy.

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