

Original Research Article

Human and environmental reservoirs of intestinal parasites in the city of Yaoundé, Cameroon: an update in the context of Covid-19 pandemic

ABSTRACT

Aims: Intestinal parasitic infections are persistent in Africa, and we questioned here whether prevention measures imposed by the occurrence of the Covid-19 pandemic in 2020 could alleviate this threat in lowlands of the city of Yaoundé, Cameroon.

Study design: We monitored the trend of intestinal parasites in human and environmental samples from the seven subdivisions of the city of Yaounde, before (November-December 2019) and during (July-August 2020, November-December 2020) the Covid-19 pandemic.

Methodology: Parasitological analysis were performed to check for the presence of helminths and protozoans in stools from inhabitants as well as in water, soil and fresh vegetables, using standard Kato Katz and Formol Ether methods. The minimum effective sample size considered for the estimation of parasite richness, parasite infection and contamination indexes was 30 for human and 30 for environmental samples (i.e. soils, water and vegetables) per location.

Results: Of the 19 parasite species identified in human and the environments, twelve were helminths and seven protozoans. The overall parasite species richness reached 16 in 2019 (12 helminths and 04 protozoans) and 19 in 2020 (12 helminths and 07 protozoans), with about 62.5-68.4% of species shared by human and environments. The parasite frequencies in human (21.03%) and water/soils (32.3%) in 2019 did not differ statistically with those of 2020 (14.6-20.3% and 10.8-35.4%,

respectively). The contamination rate of vegetables (i.e. carrots, lettuce, basil, celery, etc.) has increased from 2019 (6.1-9.1%) to 2020 (9.1-24.2%), and was frequently due to roundworms, hookworms, *Entamoeba* and *Cryptosporidium* cysts. The findings suggest persistent risk associated with intestinal parasite irrespective to measures imposed by Covid-19 in study locations.

Conclusion: The current control approaches may therefore integrate ecological epidemiology of the intestinal parasite infections as complementary strategy in African cities.

Keywords: Intestinal parasites; human; soils; water; vegetables; Covid-19; Yaoundé.

1. INTRODUCTION

Environmental parasitic diseases are emerging health problem in sub-Saharan African countries. Among them, there are some Neglected Tropical Diseases (NTDs) formally considered as targets for achieving the Sustainable Development Goals (SDGs) related to ensuring healthy lives and well-being for all at all ages by 2030 [1]. These targeted parasitic diseases are schistosomiasis caused by infection with *Schistosoma* trematodes, Soil-Transmitted Helminthiasis (STHs) caused by infection with intestinal worms (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, *Ancylostoma duodenale*, *Strongyloides stercoralis*, etc.), foodborne diseases caused by trematode worms ("flukes") (*Clonorchis* spp., *Opisthorchis* spp., *Fasciola* spp., *Paragonimus* spp. and tapeworm infections i.e. taeniasis, cysticercosis and echinococcosis). The burden of such diseases is particularly substantial in human settings and includes enteric protozoan and zoonotic infections [2-4]. This observation recalls for the relevance of the One Health principle, which recognizes the reliability of human well-being with animal and environmental health.

Soil-transmitted helminths infections affect over 1.5 billion people worldwide (6,300 deaths in 2016), whereas 200 000 new infections by foodborne pathogens and 5.5 million of tapeworm infections were recorded annually. For schistosomiasis, about 236 million people required MDA and 24,000 deceased annually [1]. Moreover, protozoan infections mostly due to *Entamoeba histolytica/dispar*, *Giardia lamblia* and *Cryptosporidium* sp. contribute to the

global mortality and morbidity [5-7]. In Africa, almost 200 million people experience parasite infections caused by roundworms (*A. lumbricoides*), hookworms (*A. duodenale*, *N. americanus*) and whipworms (*T. trichiura*) [8]. They are more prevalent in cities where anarchic urbanization is associated with a poor sanitation, poverty, low access to potable water, poor personal hygiene, overcrowding and other social issues [4, 7]. Without environmental interventions to interrupt transmission, human reinfection is common and may cause various disorders (diarrhoea, pains, malnutrition, anaemia, etc.).

Apart from environmental factors (soils, water, etc.), intestinal parasites are transmitted to human through plants, fruits and vegetables [9]. In Cameroon, the environments and gardening products contribute in the spreading of gastrointestinal parasites infections in urban and rural locations [10-15]. Agricultural activities increase the health risk associated with intestinal parasite infections, among other factors such as gender, absence of chemical prophylaxis, personal hygiene and presence of animals [16, 17]. Based on the old hypothesis that children from 4 to 15 years are the most affected, the control normally involves periodic administration of antihelminthic drugs to children at school or through community based distribution. However, the relative distribution of enteric parasite infections within all age groups suggests a systematic revision of the current control approaches of soil-transmitted parasitic infections [17]. The rapid assessment of the first global roadmap for intervention on NTDs launched since 2012 reached only 7% of progress towards the elimination of soil transmitted helminths as a public health problem in 2020 [1, 18]. The re-defined WHO roadmap for Neglected tropical diseases therefore shifts to more collaborative and cross-sectorial approaches to achieve some key operational targets among which 75% reduction of Disability adjusted life-years and 100% access to water, sanitary, and hygiene in endemic zones.

The implementation of such cross-sectorial approaches in the context of Covid-19 outbreak included the increase of WASH principles and activities for the prevention of infections, especially in Africa where access to effective treatments and vaccines was limited. This

situation is critical for several thousands of people deprived from access to improved drinking water sources, hygiene and sanitation, especially in lowlands extensively used for agriculture and/or market garden activities. Here, we have tested the hypothesis that trend of intestinal parasite burden in lowlands with poor hygiene and sanitation standards might be mitigated by prevention measures and actions promoted within communities against the Covid-19 pandemic. In accordance with the global objective of the “One Health” approach and the measures articulated in the Global Vector Control Response by 2030 [19], this paper is questioning the ecological relationships between human reservoirs of intestinal parasites and environmental settings in main African cities.

2. MATERIAL AND METHODS

2.1 Study area

The study took place in the capital city of Cameroon, Yaoundé (3°51'N; 11°30'E). The city area of nearly 3 million inhabitants in 2020 belongs to the Mfoundi administrative division and covers ~ 200 Km² [20]. The study area belongs to the equatorial climatic domain and the river Mfoundi and its tributaries forms the main hydrographic network. Study participants were recruited across the seven subdivisions of the city. We selected 13 sampling locations based on their distribution in the seven subdivisions, and the presence of lowland areas, streams and/or market gardening activities. They were Nkolondom and Etoa Meki (Yaoundé I), Tsinga and Cité verte (Yaoundé II), Efulan and Ngoa Ekelle (Yaoundé III), Ekounou and Nkodengui (Yaoundé IV), Essos (Yaoundé V), Mvog Betsi and Etoug Ebe (Yaoundé VI), Nkolbisson and Oyom Abang (Yaoundé VII) (Figure 1).

2.2 Participants

Participants were all asymptomatic individuals living in the seven selected subdivisions.

They were recruited through a non-random selection of 30 households per location among which at least one participant was enrolled per period. The “minimal effective population size” expected across the seven subdivisions per study period was 390 participants, representing 1.3 per 10.000 inhabitants of the targeted population. Each subject

Comment [A1]: Age of the participants kindly mention

Comment [A2]: Why were these locations selected and in which time of the year

Comment [A3]: Permission fro local authorities?

Comment [A4]: Kindly explain the inclusion and exclusion criteriain detail

Comment [A5]: Name of location

Comment [A6]: Definition of targetted population in this study ?

(tutors/parents of children under 5 years, age group of 6-15 years and adults) provided formal approval for their participation to the study and received a free anti-parasitic treatment in case of positive result. Local authorities also approved the study.

Comment [A7]: Kindly elaborate each

Comment [A8]: Name of the authority

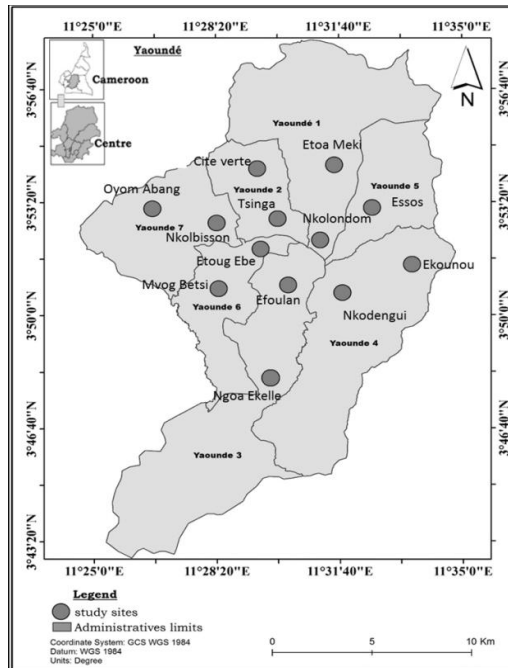


Figure 1: map of the city of Yaoundé showing its subdivisions and sampling sites

Comment [A9]: Sampling sites in map is 13 in number ?

2.3 Sampling Procedures

2.3.1 Stool collections

Participants gave their stools on a voluntary basis. The stool container provided to each participant one day before while explaining the faecal sampling procedure allowed the collection of faecal samples the next day between 8:00 to 10:00 am. Samples were then stored accurately until parasitological procedures in laboratory.

Comment [A10]: Gave their sample at which place

Comment [A11]: Who explained the procedure ?

Comment [A12]: ? am/pm

Comment [A13]: At what temperature and where were the samples stored ?

2.3.2 Environmental Samples

Comment [A14]: Name of the laboratory
How were the samples processed ?

They consisted on water and soils extracted from wells, rivers and furrows of study locations, as well as from some plants and vegetables sold in three market places (Mfoundi, 8ème-Tsinga, Etoudi). In laboratory, we proceeded on these extracts according to Rodier et al [21].

2.3.3 Parasitological Analysis

Stool and environmental samples were checked for intestinal parasites using Kato-katz (KK) [22] and Formalin-Ether Concentration (FEC) techniques [23]. For each stool sample, we mounted 41.7 mg of fresh material under a cover slide for KK thick smears, and under another cover slide with lugol's iodine for FEC thick smears. Preparations were examined under light microscope by qualified technicians to check for the presence of intestinal parasites at 100X and 400X magnifications. The protocol used for soil samples was similar to that described above for stool samples. We adapted the method described by Ayres & Mara [24] to KK and FEC thick smear analysis with 150µL of discarded water samples from environments and vegetables. A slide was "positive" or "negative" based on the crosschecking examination of KK and FEC thick smears, known to be highly sensitive for helminths and protozoans [25].

2.4 Data Analysis

The overall parasite load (N) in stools/soil samples, expressed as the total number of parasites (eggs/larvae) per gram of sample was estimated following the WHO formula i.e. $N = nx24$, with n as the number of parasites counted in thick smear (41.7 mg of pellet). The overall parasite load (N) of a single water sample (150µL) discarded from environments and vegetable (approximately twice of the size of template used in solid samples) was adjusted in egg per gram using the following formula i.e. $N = 2x(nx24)$. We compared parasite frequencies by locations and environments using Chi-square test, mean parasite densities with ANOVA and t-students, at 0.05 level of significance.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Parasite infestation in Human

Comment [A15]: Kindly explain how many wells , how many rivers and how many furrows

Who was trained to collect the samples

Who selected the sites and on what basis sites were selected

Local authority approval

When were the samples collected

Which month and relevance of the sites to parasite infestation

How many people get infested with these parasites in yaounde each year ?

Comment [A16]: Name of each plant each vegetable

How old were the vegetable

Source of vegetable growth

Comment [A17]: Kindly explain both procedures in brief

Comment [A18]: Working i which department or institution

Comment [A19]: Why Head of the department was not involved or senior designation staff for cross verification ?

Comment [A20]: Kindly provide photo of the species found in the study

In total, 1,169 human samples were analysed across the three periods. They included 673 male and 496 female participants (1.3 ratio), with 22.5±17.6 mean age ranged from 1 to 90 years old. We defined four age groups: <5 years (N=151: 12.9%), 6-15 years (N=393: 33.6%), 16-50 years (N=517: 44.2%) and >50 years (N=108: 9.2%). The overall prevalence did not vary between 2019 and 2020 for helminths (p=0.11), but increased for protozoans from 0.5% to 10.3%, respectively (P< .001). The helminth co-infection rate was 3.3% in 2019 and 2.3%-3.6% in 2020, while co-infections by protozoans varied from 0.0 to 0.5-1.8%, respectively. Otherwise, 2.6-2.8% of samples showed mixed helminth-protozoan infections. Parasite prevalence also showed slight variations between female and male participants irrespective of periods and parasite group. By age, the [16-50] range showed the highest prevalence (30.3%), followed by [6-15] (20.1%), 50 years and more (16.7%) and less than 5 years old (10.6%). Moreover, parasite prevalence changed according to the living places, with high frequencies of helminths in Yaoundé V (30%), IV (23.3%), VII (21.7%) compared with moderated loads (13-19%) in other subdivisions of the city (Table 1).

Table 1: Prevalence of helminths and protozoans (± 95%SD) in human samples collected in 2019 and 2020 from the city of Yaoundé, Cameroon

Variables	Nov-Dec 2019		July-August 2020		Nov-Dec 2020			
	Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans		
Sex	Females		54/229 (23.6±2.9)	0/229	34/225 (15.1±2.4)	30/225 (13.3±2.3)	47/219 (21.5±2.8)	19/219 (8.3±1.9)
	Males		28/161 (17.4±3.0)	2/161 (1.2±0.9)	23/165 (13.9±2.7)	17/165 (10.3±2.4)	32/170 (18.8±3.0)	11/170 (6.5±1.9)
Age (years)	< 5		5/36 (13.9±6.0)	0/36	6/59 (10.2±4.1)	8/59 (13.6±4.6)	5/56 (8.9±3.9)	4/56 (7.1±3.6)
	6-15		23/135 (17.0±3.3)	1/135 (0.7±0.8)	23/116 (19.8±3.8)	10/116 (8.6±2.7)	33/142 (23.2±3.6)	10/142 (7.0±2.2)
	16-50		45/180	0/180	25/182	26/182	35/155	13/155

Comment [A21]: Kindly mention months each time samples were collected

Comment [A22]: Since the study deliberately included subjects knowing their demography age and gender this is not a new finding

Comment [A23]: Mean age range is too large

Comment [A24]: Why such a wide range for subject selection kindly explain

Comment [A25]: Kindly distribute results for 2019 and 2020 separately

Comment [A26]: This is because samples were collected at different seasons

Comment [A27]: Which helminth was found, no where a single species is mentioned

Comment [A28]: Month range needs to be specified

Comment [A29]: Month range mention please

Comment [A30]: Which year is this finding?

Comment [A31]: Which parasite be specific

Comment [A32]: This age range itself is high as compared to other age range

Comment [A33]: This is because the number of subjects itself is low in order

Comment [A34]: Contrary to earlier statement that infestations are more in younger age group, kindly explain

Comment [A35]: Were the samples collected from the same subjects at three different time zone? Or different samples from different subjects ?

Variables	Nov-Dec 2019		July-August 2020		Nov-Dec 2020	
	Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans
> 50	(25.0±3.3)	-	(13.7±2.6)	(14.3±2.6)	(22.6±3.4)	(8.4±2.2)
	9/39	1/39	3/33	3/33	6/36	3/36
	(23.1±7.0)	(2.6±2.7)	(9.1±5.2)	(9.1±5.2)	(16.7±6.5)	(8.3±4.8)

Table 1 (continued):

Variables	Nov-Dec 2019		July-August 2020		Nov-Dec 2020	
	Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans
<i>Sub-divisions</i>	14/60	0/60	5/60	13/60	7/59	3/59
I	(23.3±5.6)	-	(8.3±3.7)	(21.7±5.5)	(11.9±4.3)	(5.1±3.0)
II	11/60	1/60	7/60	4/60	8/60	4/60
	(18.3±5.2)	(1.7±1.6)	(11.7±4.3)	(6.7±3.3)	(13.3±4.5)	(6.7±3.3)
III	9/60	0/60	7/60	4/60	12/60	4/60
	(15.0±4.7)	-	(11.7±4.3)	(6.7±3.3)	(9.1±5.3)	(6.7±3.3)
IV	15/60	0/60	12/60	9/60	15/60	4/60
	(25.0±5.8)	-	(9.1±5.3)	(15.0±4.7)	(25.0±5.8)	(6.7±3.3)
V	10/30	1/30	9/30	1/30	8/30	2/30
	(33.3±9.0)	(3.3±3.4)	(30.0±8.7)	(3.3±3.4)	(26.7±8.4)	(6.7±4.7)
VI	11/60	0/60	10/60	6/60	13/60	5/60
	(18.3±5.2)	-	(16.7±4.9)	(10.0±4.0)	(21.7±5.5)	(8.3±3.7)
VII	16/60	0/60	7/60	12/60	16/60	9/60
	(26.7±5.9)	-	(11.7±4.3)	(9.1±5.3)	(26.7±5.9)	(15.0±4.7)
Overall	82/390	2/390	57/390	47/390	79/389	30/389
	(21.0±2.1)	(0.5±0.4)	(14.6±1.8)	(12.6±1.7)	(20.3± 2.0)	(8.0±1.4)

SD: Standard Deviation

On the 18 parasite species identified from human, twelve were helminthes (*Ascaris lumbricoides*, *Hymenolepis nana*, *Trichuris trichiura*, *Schistosoma mansoni*, *Paragonimus westermanii*, *Necator americanus*, *Fasciola hepatica*, *Taenia* sp., *Enterobius vermicularis*,

Strongyloides stercoralis, *Schistosoma* sp. and *Trichostrongylus* sp.) and six were protozoans (*Entamoeba histolytica/dispar*, *Cryptosporidium* sp., *Balantidium coli*, *Idamoeba butschilii*, *Sarcocystis* sp. and *Giardia lamblia*) (Figure 2). The overall species richness did not differ significantly from 2019 (13) to 2020 (13-15), by sex and age groups. Species found associated together in multiple infections were *A. lumbricoides*, *H. nana*, *T. trichiura* and *S. mansoni* for helminths, and *E. histolytica/dispar*, *B. coli* and *Cryptosporidium* sp. for protozoans.

Comment [A36]: Kindly confirm from figure 2

The overall median parasite load decreased from 120 parasites/g in 2019 (helminthes: 24-48,240 p/g; protozoans: 24 p/g) to 24-48 parasites/g (helminthes: 24-85,176 p/g; protozoans: 24-960 p/g) in 2020. However, the trend of median protozoan density did not differ between sampling periods, neither by sex nor by age groups. Otherwise, the intensity of helminth infection was high throughout periods compared to protozoan load, with the overall mean of 1875.5 versus 24 p/g in November 2019, 790.4 versus 83.8 p/g in July-August 2020 and 2112.3 versus 35.9 p/g, respectively.

Comment [A37]: Now that is a huge decline

Comment [A38]: Which month and year ?

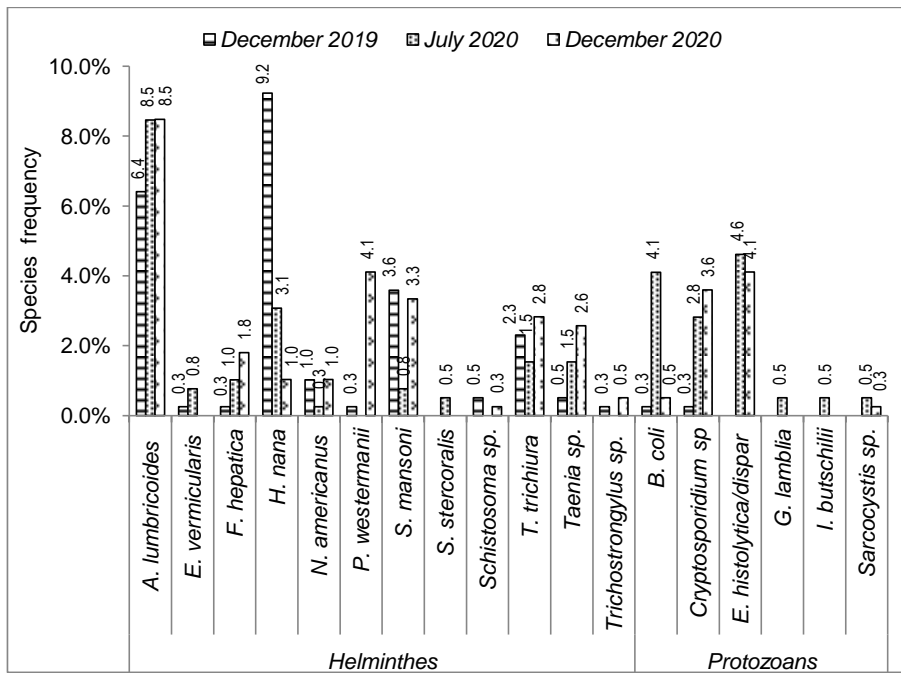


Figure 2: Frequencies of helminth and protozoan species identified in human samples in 2019 (November-December) and 2020 (July-August, November-December)

3.1.2 Environmental parasite contamination

In total, 489 environment samples were collected throughout the 3 periods (N=163 for each period, i.e. water=65; soils=65; vegetables=33). The overall contamination rate ($\pm 95\%SD$) in water and soils did not vary significantly between 2019 ($32.3 \pm 4.1\%$) and 2020 ($23.1 \pm 2.7\%$) for helminths ($P=0.07$), and for protozoans ($33.1 \pm 4.2\%$ versus 36.2 ± 3.0 , respectively; $P=0.63$) (table 2).

Table 2: Frequency of helminths and protozoans ($\pm 95\%SD$) in environment samples and vegetables collected in 2019 and 2020 from the city of Yaounde, Cameroon

Nov-Dec 2019		July-August 2020		Nov-Dec 2020	
Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans

Water	30/65 (46.2±6.4)	28/65 (43.1±6.3)	7/65 (10.8±4.0)	31/65 (47.7±6.4)	21/65 (32.3±6.0)	32/65 (49.2±6.4)
Soil	12/65 (18.5±4.9)	15/65 (23.1±5.4)	7/65 (10.8±4.0)	12/65 (18.5±4.9)	25/65 (38.5±6.2)	19/65 (29.2±5.8)
Water+Soils	42/130 (32.3±4.1)	43/130 (33.1±4.2)	14/130 (10.8±2.8)	43/130 (33.1±4.2)	46/130 (35.4±4.2)	51/130 (39.2±4.3)
Vegetables	3/33 (9.1±5.2)	2/33 (6.1±4.3)	8/33 (24.2±7.8)	3/33 (9.1±5.2)	8/33 (24.2±7.8)	8/33 (24.2±7.8)

SD: Standard Deviation

From discarded water of vegetables, the overall contamination rate reached 15.2±6.5% and 31.8±5.9% in 2019 and 2020, respectively ($P= 0.13$). This rate varied by type of vegetables with a significant number of vegetables contaminated in 2020 (i.e. carrots, basil, lettuce, cabbage, celery, wild basil, African nightshade, parsley, leek, waterleaf and jute (Table 3).

Table 3: Occurrence of helminths and protozoans in vegetables sold at market places of Yaounde, in 2019 and 2020.

Common plants (local names)	Scientific names	Nov-Dec 2019		July-August 2020		Nov-Dec 2020	
		Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans
Basil	<i>Ocimum basilicum</i>	+	-	+	-	+	+
Carrot	<i>Daucus carota</i>	+	-	+	+	+	-

Comment [A39]: Kindly split the two results year wise according to the finding

Table 3 (continued)

Common plants (local names)	Scientific names	Nov-Dec 2019		July-August 2020		Nov-Dec 2020	
		Helminths	Protozoans	Helminths	Protozoans	Helminths	Protozoans
Celery	<i>Apium graveolens</i>	-	+	+	-	-	+
Cabbage	<i>Brassica oleracea</i>	-	-	-	-	+	+
Lettuce	<i>Lactuca sativa</i>	+	-	+	-	-	+
Wild basil (messepe, etc.)	<i>Clinopodium vulgare</i>	+	+	+	+	+	+
African nightshade (zom, jamajama.)	<i>Solanum scabra</i>	-	-	-	-	+	+
Parsley	<i>Petroselinum crispum</i>	-	-	+	-	-	-
Leek	<i>Allium porrum</i>	-	-	+	-	+	+
Waterleaf	<i>Talinum triangulare</i>	-	-	+	+	-	-
Jute mallow (tegue, keleng keleng")	<i>Corchorus olitorius</i>	-	-	-	-	+	-

Caption: - : negative; +: positive

In water and soil samples, the species richness was 16, including eleven helminthes (*A. lumbricoides*, *H. nana*, *T. trichiura*, *S. mansoni*, *P. westermanii*, *N. americanus*, *F. hepatica*, *Taenia* sp., *E. vermicularis*, *S. stercoralis* and *Schistosoma* sp.) and five protozoans (*E. histolytica/dispar*, *Cryptosporidium* sp., *B. coli*, *I. butschilii* and *E. coli*). The species composition showed variations between 2019 (13 species) and 2020 (14 species), between water (5-12 species), soils (8-12 species) and vegetables (3-8 species) (Table 4).

Table 4: Helminth and protozoan species composition and relative frequency in soils, water and vegetables collected in 2019 and 2020 from the city of Yaounde, Cameroon

	Nov-Dec 2019			July-August 2020			Nov-Dec 2020		
	Soils	Water	Vegetables	Soils	Water	Vegetables	Soils	Water	Vegetables
Helminths									
<i>A. lumbricoides</i>	+++	++++	+++	++	-	++	++++	++	+++
<i>E. vermicularis</i>	++	++	-	++	++	-	++	-	+++
<i>F. hepatica</i>	++	+++	-	++	-	-	++	++	++
<i>H. nana</i>	++	++	-	-	++	-	++	+++	-
<i>N. americanus</i>	++	+++	-	-	-	++	-	++	-
<i>P. westermanii</i>	++	++	-	-	-	-	-	-	-
<i>S. mansoni</i>	-	++	-	-	-	-	++	+++	-
<i>S. stercoralis</i>	+++	++	++	++++	+++	++++	++++	++++	++++
<i>Schistosoma sp.</i>	-	-	-	-	-	-	-	++	-
<i>T. trichiura</i>	++	++	-	++	-	-	++	-	-
<i>Taenia sp.</i>	++	++	-	-	-	-	-	-	-
Protozoans									
<i>E. histolytica/dispar</i>	++++	++++	+++	++++	++++	++	++++	++++	++++
<i>Cryptosporidium sp.</i>	-	-	-	++	++	+++	++	++	+++
<i>B. coli</i>	++	-	-	-	-	-	++	+++	-
<i>I. butschilii</i>	++	++	-	-	-	-	++	++++	-
<i>E. coli</i>	-	-	-	++	-	-	-	++	+++

Caption: - : absent; frequency: +, below 1% ; ++, 1-5%; +++, 5-10%; +++++, above 10%

The median abundance of helminths and protozoans was globally comparable between 2019 and 2020 amongst soils, water and vegetables (48-96 parasites/g). However, the

arithmetic mean showed significant discrepancies between helminths and protozoans, varying in water samples (80.52 vs. 5792 p/g in 2019, 48.00- 66.29 vs. 229.16-53.00 p/g in 2020), in soils (44.00 vs. 40.00 p/L in 2019, 113.00- 67.20 vs. 100.0 - 95.50 p/g in 2020) and in vegetables (128.00 vs. 120.00 p/g in 2019, 102.00-78.00 vs. 48.00- 54.00 p/g in 2020), respectively).

3.2 Discussion

We have tested here the hypothesis that the trend of intestinal parasite burden in lowlands of the capital city of Cameroon might be mitigated by an increased community-based prevention measures and actions imposed by the occurrence of the Covid-19 pandemic. In this research, we addressed the human and environments as potential parasite reservoirs of intestinal parasite infections. The combination of conventional parasitological methods allowed the morphological identification of intestinal parasites in human stools and environmental samples (soils, water and fresh vegetables) under a standard microscope. The same methodological approach had been successfully adapted in the field for direct parasite identification in environmental samples such as water and soils [15].

The first main outcome of the study is the persistence of the risk associated with intestinal parasite infection across the thirteen study locations in 2019 and 2020. This suggests that the occurrence of the Covid-19 in 2020 did not significantly affect the trends of parasite frequency and species richness compared with 2019, as expected with the extensive implementation of Covid-19 prevention measures like hand washing with soap or antiseptic solutions. The parasitological examination of human and environmental samples collected from these specific areas revealed the presence of harmful helminth and protozoan parasites, mostly involved in diarrhoea and intestinal disorders in human. The parasite composition in human samples was dominated by helminths (with frequency more than 1%), especially roundworms (*A. lumbricoïdes*, *S. stercolaris*, *E. vermicularis*), tapeworms (*H. nana*, *Taenia* sp.), hookworms (*N. americanus*), whipworms (*T. trichiura*) and flukes (*Schistosoma* sp. *F. hepatica* and *P. westermanni*), as well as by protozoans i.e. *E.*

Comment [A40]: Kindly elaborate the connection between preventive measure during covid and increase in parasite

Comment [A41]: Kindly share photograph of each parasite found during the study

histolytica/dispar and *Cryptosporidium* sp. The trends of parasite richness and frequency (~20% for helminths, 0.5-12.6% for protozoans) have shown variations throughout age groups. According to the WHO classification for STH risk [26], our study locations might be ranked into moderate to high-risk ($\geq 20\%$), where recommends periodic mass deworming to at-risk people, administered at least once a year, when prevalence is more than 50%, deworming should be administered twice a year [27]. The trends of intestinal parasite burden are consistent with what has been previously reported from the study location [28], across the country [12, 13, 28-36] and elsewhere in tropical Africa [7, 37-39].

The second major outcome of our study confirmed the fact that all age groups are subjected to intestinal parasite infections, even though the current deworming programs in Cameroon and endemic countries are essentially limited to school-aged children [29, 40]. We observed the highest prevalence of intestinal parasite infections among age groups other than school-aged children, as previously noticed in West Cameroon [17, 41]. This finding is an indication that all people living in lowlands of Yaoundé have similar exposure risk, through direct contact with contaminated environments (water or soils) or by adopting wrong hygiene and sanitation like the consumption of street foods and non-systematic hand-washing practices. Our study provided further evidences that soils, water and vegetables from study locations are potential sources of dissemination of intestinal parasite infections, especially caused by helminths and protozoans. Except for *Trichostrongylus* sp. *Sarcocystis* sp. and *G. lamblia* found only in human samples, the protozoan and helminth composition in environmental samples was quite similar to that observed in human from study locations. Similar results were previously reported in the same locations [14, 15, 42, 43], across the country [32, 34, 44, 45] and elsewhere in Africa [4, 7, 39] and Brazil [46]. This context falls into the one health approach, which has been adopted worldwide to address public health threats linking human, animal hosts and their environmental interfaces [47]. Based on their presence in human, water surfaces and soils, the identified pathogens can be therefore classified as "ubiquitous" parasites [48].

Comment [A42]: Full form please

Comment [A43]: How many subjects in the study recieved deworming drugs ?

Finally, the study has emphasized health risks associated with the consumption of fresh vegetables distributed in markets of high attendance (Mfoundi, 8eme, Etoudi). We provided further evidence that inhabitants of Yaounde are exposed to intestinal parasite infections through the consumption of fresh vegetables sold at main market places. Fresh vegetables (Wild basil, carrot, lettuce, celery, leek, etc) used for various gastronomic dishes showed high contamination rates mainly by *A. lumbricoides*, *E. vermicularis*, *S. stercoralis*, *Entamoeba* spp. and *Cryptosporidium* sp. This observation is not an isolated case, although an increased health risk had been previously found associated with the consumption of street foods, beverages and drinking water [49-52]. Additional studies have demonstrated that biological contamination can be extended to other groups of waterborne pathogens including viruses and bacteria [53]. Factors enhancing parasite contamination across study sites are essentially associated with lack of individual hygiene and low sanitation standards. For example, traditional toilets used by local communities communicated directly with rivers and anarchic occupation of surrounding lands for farming and/or livestock activities might not guarantee safe and healthy environments for residents, especially with the occurrence of outbreaks such as Covid-19, cholera, etc.

4. CONCLUSION

Our findings suggest that intestinal parasites causing enteric diseases are widely distributed in human and environments of the study locations, and routes for parasite transmission to human through contaminated water, soils and fresh vegetables. Intestinal parasite burden was critical at all age groups and the increased of individual preventive measures imposed by the occurrence of the Covid-19 pandemic at community level did not mitigate the trend of helminth and protozoan frequency in study sites. Furthermore, the health status of inhabitants from the study locations associated with deprived environments and inadequate sanitation standards are among key factors that explain the persistence of soil-transmitted and other foodborne diseases. An integrated management of ecological relationships

between host reservoirs of intestinal parasites, household practices and occupations in marshy environments should be therefore considered in African cities.

CONSENT AND ETHICAL APPROVAL

All authors declare that informed consent was obtained from the participants, and hereby declare that all protocols for stool collections have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki." The Centre Region Ethical Committee of Cameroon approved the study through an ethical clearance N° 200-1/CRERSHC/2019 delivered on the 8th February 2019 for year 1 (2019), and a renewed clearance CE N°190-1/CRERSHC/2020 delivered on the 13th January 2020 for the study extension in 2020.

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