

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), Efoulan 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

(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.

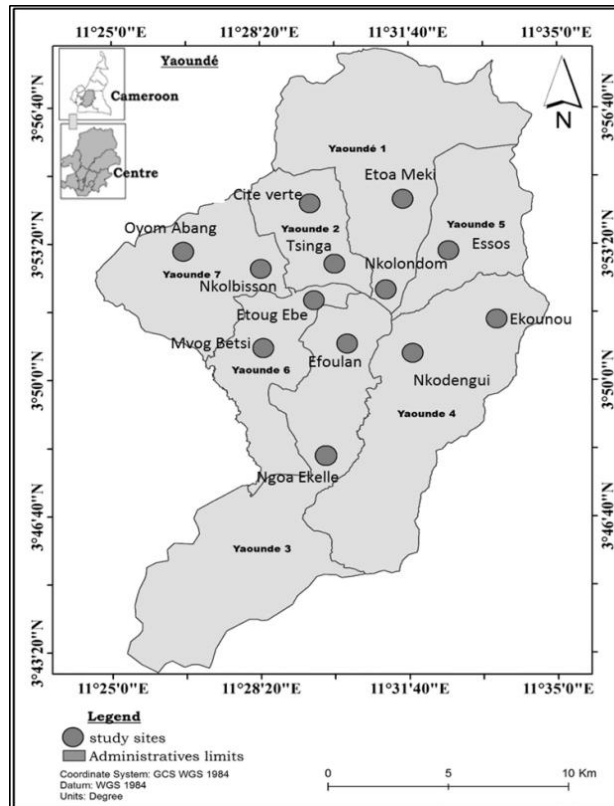


Figure 1: map of the city of Yaoundé showing its subdivisions and sampling sites

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.

2.3.2 Environmental Samples

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

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 ($\pm 95\%SD$) 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 \pm 2.9)	0/229	34/225 (15.1 \pm 2.4)	30/225 (13.3 \pm 2.3)	47/219 (21.5 \pm 2.8)	19/219 (8.3 \pm 1.9)
	Males	28/161 (17.4 \pm 3.0)	2/161 (1.2 \pm 0.9)	23/165 (13.9 \pm 2.7)	17/165 (10.3 \pm 2.4)	32/170 (18.8 \pm 3.0)	11/170 (6.5 \pm 1.9)
Age (years)							
	< 5	5/36 (13.9 \pm 6.0)	0/36	6/59 (10.2 \pm 4.1)	8/59 (13.6 \pm 4.6)	5/56 (8.9 \pm 3.9)	4/56 (7.1 \pm 3.6)
	6-15	23/135 (17.0 \pm 3.3)	1/135 (0.7 \pm 0.8)	23/116 (19.8 \pm 3.8)	10/116 (8.6 \pm 2.7)	33/142 (23.2 \pm 3.6)	10/142 (7.0 \pm 2.2)
	16-50	45/180	0/180	25/182	26/182	35/155	13/155

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.

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.

