Research Article

Universal Journal of Life and Environmental Sciences **2024, Vol 6, Pages 158-165 Serie 1**

Submission (17 May 2024) Accepted and Published (December 2024) www.ijarme.org

Distribution of infectious eggs and larvae of helminthes in stools and soil in the medical district of Awae in the city of Yaounde, Cameroon

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Abstract

With the aim of studying the distribution of helminths in an urbanized tropical environment, a study of stool and soil samples was carried out from May to September 2023. Stool and soil samples from household waste discharge points were examined. The Lugol fresh state technique allowed us to search for the vegetative and dissemination forms of parasites, Kato-katz was specific to helminths; helminths, the most abundant were Strongyloides stercoralis (11.42%). No statistical association between age or sex and intestinal parasites ($p > 0.05$) was observed. . Parasitic forms were observed in 73% of soil samples. the most abundant were Strongyloides stercoralis (26.70%), Ascaris lumbricoides (18.49%). In the soil samples, we observed parasites also present in humans. In this study, we found that poor sanitary conditions are the cause of the presence of intestinal parasites and we demonstrated that contaminated soil plays a role in the infection and reinfection of the population.

Key words : Egg, Feces, Helminthes, Larvae, and Soil

Résumé

Dans le but d'étudier la distribution des helminthes en milieu tropicale urbanisée, une étude des échantillons des selles et du sol a été menée de mai à septembre 2023. Les échantillons des selles et du sol provenant des points des décharges des ordures ménagères ont été examinés. La technique de l'état frais au lugol nous a permis de rechercher les formes végétatives et de dissémination des parasites, Kato-katz était spécifique aux helminthes ; les helminthes, les plus abondants étaient *Strongyloides stercoralis* (11,42%). Aucune association statistique entre l'âge ou le sexe et les parasites intestinaux ($p > 0,05$) n'ont été observés. . Des formes parasitaires ont été observées dans 73% des échantillons du sol. Les plus abondants étaient *Strongyloides stercoralis* (26,70%), *Ascaris lumbricoides* (18,49%). Dans les échantillons de sol, nous avons observés les parasites également présents chez les humains. Dans cette etude, nous avons constaté que les conditions sanitaires déficientes sont à l'origine de la présence des parasites intestinaux et nous avons mis en évidence que le sol contaminé joue un rôle dans l'infection de la population.

Mots clés : Gastro-entérites, Helminthe, Selles et Sol, Yaounde

I.INTRODUCTION

Intestinal helminthes are among the most common infections worldwide, with more than 1.5 billion people, or nearly 24% of the world's population, being infected globally. These infections affect the poorest and most disadvantaged communities with limited access to drinking water, sanitation and hygiene in tropical and subtropical regions, with the highest prevalence recorded in sub-Saharan Africa, China, South America and Asia, World Health Organization (1997), World Health Organization (1998). They are transmitted by eggs present in human excrement, which contaminate the soil where sanitation conditions are insufficient. More than 260 million preschool children, 654 million school-age children, 108 million adolescent girls and 138.8 million pregnant or lactating women live in areas where there is widespread transmission of these parasites and require treatment and preventative measures. It is estimated that more than 600 million people worldwide are infested with *S. stercoralis*. However, since this parasite is also transmitted in areas where sanitation is poor, its geographic distribution largely coincides with that of other soil-transmitted helminthiases World Health Organization (1994).

The main species responsible for the disease in humans are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*). STHs caused by these different species are generally grouped in the same category because they are diagnosed in a similar way and respond to the same medications. *Strongyloides stercoralis* is an intestinal helminth with particular characteristics: its diagnosis requires different methods from those used for other STHs and it therefore often escapes detection. Furthermore, this parasite is not sensitive to albendazole and mebendazole and therefore cannot be combated as part of mass preventive campaigns targeting other geohelminthiases. Geohelminths are transmitted by eggs excreted in the stools of infested people. Adult worms live in the intestines, where they lay thousands of eggs every day. In places where sanitation means are insufficient, these eggs contaminate the soil. Transmission can take place according to different scenarios: eggs present on vegetables are ingested when these vegetables are consumed without having been properly cooked, washed or peeled; eggs are ingested when consuming contaminated water; and eggs are ingested by children who play on contaminated soil and put their hands in their mouths without washing them.

In addition, hookworm eggs hatch in the soil according to physicochemical properties, Rodier, 2009 releasing larvae which continue their development until reaching a stage where they are able to actively pass through the skin. World Health Organization (1997), World Health Organization (1998). Most cases of hookworm infestations occur when a person walks barefoot on contaminated soil. There is no direct human-to-human transmission, nor infestation from fresh stools, because the eggs excreted in the stools must mature for approximately 3 weeks in the soil before acquiring infectious power, Ajeagah 2017. Since the worm's *A. lumbricoides*, *T. trichiura* and hookworms do not multiply inside the human body, reinfection can only take place the outcome of contact with larvae at an infective stage in the environment.

S. stercoralis can reproduce in the host and in immunocompromised individuals; if this multiplication is not controlled, it can be fatal World Health Organization (1994). Board for the diagnosis of intestinal parasites. Geneva. 29p. Indeed, given the precarious living conditions of the populations of certain neighborhoods, Yaoundé would be an interesting area with an increased level of unsanitary conditions because with the rapid growth of the population, its inhabitants find themselves occupying deprived neighborhoods of the city characterized essentially by their under-equipment and under-integration (Mongoue et al., 2021) favorable to the maintenance of the infesting forms of the parasites responsible for parasitic gastroenteritis. It is with this observation that we undertook this work, the main objective of which is to study the distribution of environmental forms of enteroparasites in the District of Yaoundé 4 and the relationship of these with cases of gastroenteritis. More precisely, it is a question of: - Isolating and identifying the different species of enteric parasites in the stools of patients visiting a medical center and at garbage disposal points in the Awae district; - Analyzing the existing relationship between environmental variables and the transmission of enteroparasites in Awae.

II: MATERIALS AND METHODS

II.1. Study area

Our study area is located in the city of Yaoundé, located in the forest region of the South Cameroonian plateau. Our descriptive cross-sectional study involved 100 stool samples from patients presenting to the Reaven Medical-Surgical Clinic, with or without clinical manifestations of gastroenteritis, and 100 soil samples. Each patient received a dry, clean, transparent container with a wide opening and tightly closed for collecting freshly passed stools either at home or at the Clinic. The examination at the Clinic laboratory level was carried out on freshly emitted fecal matter. For each stool sample, two examinations were carried out, namely: a macroscopic examination and examination using the IVYMEN brand binocular optical microscope (Rousset, 1993). The results were recorded in a notebook. the existence of added elements which may be of parasitic origin (Tænia rings, pinworm adults, roundworm adults) or non-parasitic (blood, mucus, dietary fiber) (Kadiri, 2010).

II.2. Microscopic examination of stools

Using an applicator stick (match or toothpick), take a small portion of stool sample and mix with the drop of physiological saline then, in the same way, take a little stool and mix with the Lugol drop to make an iodine preparation (if applicator sticks are used, throw them away) subsequently, cover the drop of physiological saline and the drop of iodine with a cover slip, to this, hold the coverslip inclined in contact with the blade touch the edge of the drop and gently lower the coverslip, this will avoid air bubbles in the preparation. Finally, examine the preparations at 10 x magnification and then at 40 x magnification, proceeding systematically (from top to bottom or from right to left) so as to observe the entire preparation. The Kato-Katz technique described by

Katz et al in 1970 is a technique used to search for helminth eggs in stools.

Indeed, a fragment of saddle is taken from each pot using a plastic rod and placed on a stainless steel sieve (mesh size 212Um), then pressure is exerted on it using the rod and the sifted saddle is deposited in the 41.7mg template orifice placed in the center of a slide previously labeled (identification number of the subject), the excess saddle is thus removed in shaving the surface of the template. Using forceps, a piece of cellophane paper previously soaked in Kato's solution (1 mL of 3% Malachite green + 100 mL of distilled water + 100 mL of glycerin) for at least 24 hours is taken to cover the stool. The latter is thus spread into a uniform smear by lightly rolling a test tube on the cellophane paper. The preparation thus obtained is analyzed at least 30 minutes after spreading the stool by optical microscopy at 10x then 40x magnification.

II.3. Soil biological parameters

Soil biological parameters were determined by the distilled water method by Al-khamesi (2014). For each soil sample, 3g was mixed with 10mL of distilled water. Solid particles were removed by sieving the mixture using a 180µm fine mesh sieve. The sediments of the soil samples were obtained by pouring the supernatant after 24 h. The prepared samples were processed using the modified distilled water technique. 5mL of each sediment were placed in a 15mL conical bottom tube and filled with distilled water to its limit followed by centrifugation (MINOR35 brand centrifuge) at a speed of 300 rpm for 5 minutes.

The supernatant was discarded and the pellet was homogenized using a Pasteur pipette. A drop of pellet to which 1% Lugol was added and examined between slide and coverslip using 10x then 40x objectives of an IVYMEN brand binocular optical microscope to identify parasites. The data was entered into Microsoft Excel 2016 for plotting the graphs. Concerning the statistical tests to be performed, the Kolmogorov Smirnov K normality test was used to check the normality of the data collected during this study. This test having revealed that the data do not follow a normal distribution, non-parametric tests were used to analyze the data of this study.

III.RESULTS AND DISCUSSION

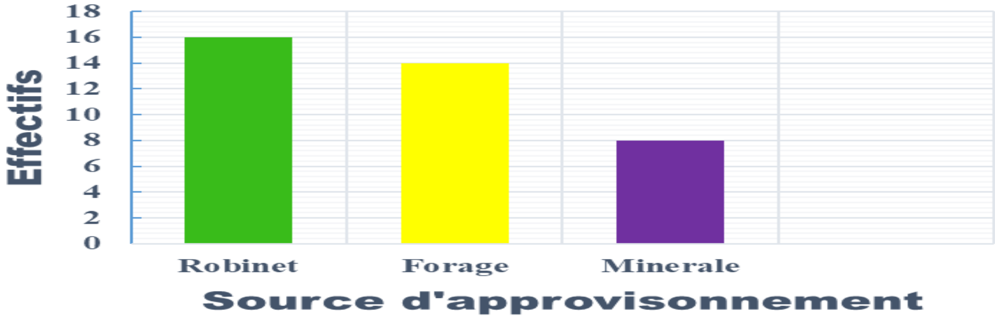
III.1. Stool examination to isolate and identify helminths

Our study carried out in the laboratory of the Reaven Medical-Surgical Clinic from May to August for 100 hospitalized or outpatient patients allowed the identification of numerous species of human intestinal parasites. Among the 38 parasitized patients, 20 were male, representing a percentage of 39.21% of the total number of men (51), while 18 female patients were parasitized, representing a percentage of 36.73% of the overall number of women, 20 male patients were parasitized, representing a percentage of 53% of the overall number of parasitized individuals, while 18 female patients were parasitized, with a percentage of 47% of the overall number of parasitized individuals.

During our study, we observed that the Binguela district contains the largest number of infected patients (8 patients), i.e. a percentage of 21.05% compared to the total number of positive cases recorded in all districts. The Awae and Montie districts are also considered major districts, the respective percentages of parasitized patients are 15.78% and 13.15% compared to the total number of positive cases recorded in all districts. The other districts including Manassa, Abang and Marie-Louise contain the fewest parasitized patients (1 per district) with a percentage of 2.63% each compared to the total number of positive cases recorded in all districts. During our study, we observed that the Binguela district contains the largest number of infected patients (8 patients), i.e. a percentage of 21.05% compared to the total number of positive cases recorded in all districts. The Awae and Montie districts are also considered major districts, the respective percentages of parasitized patients are 15.78% and 13.15% compared to the total number of positive cases recorded in all districts. The other districts including Manassa, Abang and Marie-Louise contain the fewest parasitized patients (1 per district) with a percentage of 2.63% each compared to the total number of positive cases recorded in all districts (Figure 9). During our study, parasitized patients revealed that they consumed water from three different sources (tap, borehole and mineral). The histogram shows that 16 infected patients consumed tap water, a rate of 42.10%. Then 14 infected patients consumed water from the borehole, a percentage of 36.84%. Finally, 8 infected patients consumed mineral water with a percentage of 21.05%. 17.31%. Nausea and abdominal bloating are considered among the major signs; their respective percentages are 16.35% and 11.54%. The other signs are less important, of which anal pruritus is the least common sign with a rate of 6.73% (Figure 11). Of the 38 parasitized patients, many were infected by several parasites (polyparasitism of at least 2 different species of parasites).We observed that among infected patients, 27 presented polyparasitism with a percentage of 71%, and 11 presented monoparasitism with a percentage of 29%. The different pH values obtained during the study period were constant (pH=5) in all stations and oscillate around an average of 5 ± 0.0 U.C.

The different Humidity values obtained during the study period were constant (H=100%) in all stations and oscillate around an average of 100 ± 0.00%. The electrical conductivity fluctuated from 110 µS/cm at station S1 to 1386 µS/cm at station S8 for an average of 445.40 ± 370.60 µS/cm. Statistical tests show a significant difference (P<0.05) between the electrical conductivities of the soil from the different sampling stations.

The Total Dissolved Solids (TDS) contents obtained during the study period vary between 55 mg/L (obtained at station S1) and 663 mg/L at station S8. Statistical tests show no significant difference (P>0.05) between the soil TDS of the different sampling stations (Figure 17). Among the 100 soil samples examined during our study, 73 were positive, i.e. a rate of 73%. On the other hand, 27 were declared negative, for a percentage of 27% (Figure 1 and 2). Overall infestation rate of soil samples examined during the study period. III.1.4. Distribution of parasite species in stools and in soil samples. The systematic identification of parasite species in patient stools and soil from the different study stations shows the presence of protozoa and helminthes.



III.2. Overall distribution according to parasite groups

In the stools, we note a predominance of infestation by protozoa with a rate of 76% compared to 24% of helminths (Figure 19). For the soil, we observe a predominance of protozoa at stations S1, S3, S4, S8 and S9 while in stations S2, S5, S6, S7 and S10 helminths are the most numerous (Figure 20). Chi-square was used to compare parasite prevalences between stool and soil. Cestodes (p = 0.027) and Nematodes (p < 0.0001) found in stools and soil (at the 5% threshold; df = 1), Trematodes (p = 0.40) found in stools and soil (at the 5% threshold, df = 1) Table I.

Figure : 1 Distribution of parasitized patients according to drinking water supply during our study.

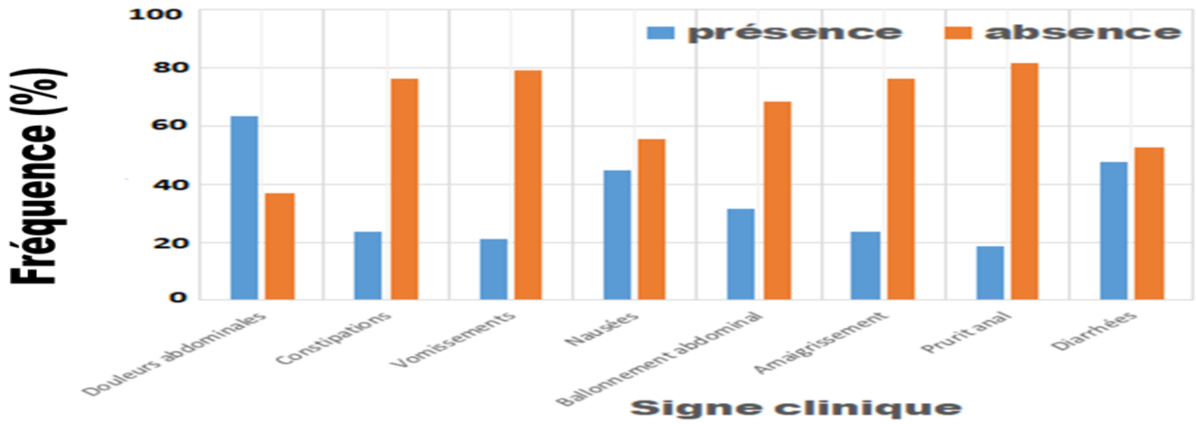


Figure 2 : Frequency (%) of monoparasitism and polyparasitism in parasitized patients during the study period.

Table I: Comparison of parasite prevalence in stools and soil according to parasite classes during the study period.

Trématodes	19 (5,29%)a	13 (2,27%)a	χ^2 = 0,69 ddl = 1 p = 0,40
Cestodes	0 (0%)a	7 (1,22%)b	χ^2 = 4,95 ddl = 1 p = 0,027
Nématodes	66 (18,38%)a	268 (46,77%)b	χ^2 = 67,23 ddl = 1 p < 0,0001

III.3. Distribution of helminth species

During our study, we observed variability in parasitic species with different rates. From the analysis of the stools, the most abundant protozoan species was Cryptosporidium spp (Tyzzer, 1907) with a number of 80. For helminths, the most abundant were the eggs of S. stercoralis with a number of 34. In the soil, E. coli was more abundant with a number of 72 for protozoa, while the larva of S. stercoralis was abundant among helminths with a number of 147.During the observations we obtained a variety of parasitic species, some of which are described in the table II and III below.

Table II: morphological characteristics of some parasitic species observed.

Œuf	Cestodes	*Hymenolepis nana*	Hyménolépiose	45-50 µm Ovale
	Nématodes	*Ankylostoma duodenale*	Ankylostomose	56-75 µm Ellipsoïdale
		Ascaris lumbricoides	Ascaridiose	45-75 µm Ovale ou rond aspect mamelonné
		Strongyloides stercoralis	Anguillulose	52-60 µm Ovale

Table III : Distribution of helminthes parasites in stool and soil

F. hepatica	19 (a) 12 (b)
A. lumbricoides	10 (a) 106 (b)
E. vermicularis	10 (a) 0 (b)
œuf anguillule	34 (a)

	6 (b)
larve S. stercoralis	7 (a) 147 (b)
Trichostrongylus spp	2 (a) 2 (b)
A. duodenale	1 (a) 7 (b)
T. trichiura	1 (a) 0 (b)
C. sinensis	1 (a) 0 (b)
H. nana	0 (a) 2 (b)
D. dentriticum	0 (a) 1 (b)
D. caninum	0 (a) 5 (b)

III.1.4. Statistical analysis

Correlations were carried out between the physicochemical variables, between the physicochemical variables and the biological variables and between the biological variables. As for the correlations between physicochemical variables, positive and negative correlations, significant and non-significant at the 1% threshold, were recorded. Temperature is correlated with conductivity (r = -0.035) and TDS (r = -0.035). Conductivity is correlated with temperature (r=-0.134), pH (r=-0.365) and humidity (r=-0.97).TDS is correlated with conductivity (r=-0.035), temperature (r=-0.201), pH (r=-0.352) and humidity (r=-0.117). Correlations were observed between physicochemical and biological variables. Temperature is correlated with *Chilomastix mesnili* (Wenyon, 1910) (r=-0.300), *Balantidium coli* (Malmsten, 1857) (r=-0.65), S. stercoralis eggs (r=-0.497) and S. stercoralis larvae (r=-0.661*). Conductivity is correlated with *A. lumbricoides* (r=0.648*) and S. stercoralis eggs (r=-0.164).The TDS is correlated with *A. lumbricoides* (r=0.624) and the eggs of *S. stercoralis* (r=-0.277) (Table IV). Correlations were also recorded at the level of biological variables between them. Thus, *E. histolytica* is correlated with *A. lumbricoides* (r=0.689*), Cryptosporidium spp is correlated with C. mesnili (r=0.818**). *Cyclospora cayetanensis* (Ortega, Gilman & Sterling, 1994) is correlated with *A. duodenale* (r=-0.014) and *Ascaris lumbricoides* is correlated with larvae of S. stercoralis (r=0.0770**) (Table V).

V DISCUSSION

To better discuss the epidemiological factors linked to the distribution of parasitic diseases, it is interesting to carry out copro-parasitological research in humans and to study the environmental and health aspects of the place where the individuals live. The vast majority of individuals studied live in conditions favorable to the proliferation of parasites (it has been observed that the populations coexist on a daily basis with Hysacam points).

In this study, we generated basic information on the prevalence of enteric parasites in the Commune of Yaoundé 4 taking into account 2 spheres: environment and human as well as their interaction. Regarding helminths and protozoa, nineteen different species were detected in the fecal samples, while seventeen different species were recovered in the environmental samples.

A total of 100 stool samples were analyzed and 38 of them were positive for intestinal parasites giving a prevalence rate of 38%. These results corroborate with studies carried out in a hospital in Biyem-Assi District and confirm the hypothesis that the global prevalence of intestinal parasites is less than 50% (Ekwale Emilia, 2019).

This is due to different factors, notably geographical and ecological, behavioral habits and living conditions of the population studied (Oyono et al., 2022); The parasitic prevalence was higher in the age group [0-10 years]. These data are similar to those of Alpha et al in 2019 but differ from the results of Ekwale Emilia in 2019 where the most infected age group was [11-15 years].

This could be explained by the fact that young children are naturally exposed because of their daily activities among others: games, contact with contaminated objects, non-compliance with hygiene measures (Alpha et al., 2019). From the analysis of stools, Helminthes (*A. lumbricoides, S. stercoralis* and *T. trichiura*) has been observed with different infection rates as in many localities in Cameroon (Oyono et al., 2022). Among the hlminths, S. stercoralis stands out by being predominant in the materials analyzed; this result is probably due to the sum of several factors that contribute to the dissemination of this group of parasites, such as the presence of animals, inadequate places to defecate and precarious wastewater.

All of these factors contribute to the penetration of S. stercoralis larvae (occasionally their consumption), most likely due to the excellent soil conditions for their proliferation, thus increasing the likelihood of human contact with the parasite (Amor & Oliveira, 2017). In contrast to the high prevalence of S. stercoralis, other helminths such as *T. trichuira, C. sinensis* and *A. duodenale* were less abundant in parasitized patients.

It was found that the population of this area lived in conditions favorable to the proliferation of parasites: houses, establishments and commercial activities were located close to Hysacam collection points, which represented risk sites harboring a variety of forms of resistance of human parasites (Nkengazong et al., 2021). Numerous studies have shown that urban soils receive contaminant loads that are generally higher in surrounding suburban or rural areas due to the concentration of anthropogenic activities, urban settlements (Ribeiro *et al.*, 2013 ; Silva *et al*., 2017 (Oguh & Eno, 2020), and in contribution to other studies (Ajeagah et al., 2018; Asi & Ajeagah, 2020), environmental parasites (spores, cysts, oocysts, eggs and larvae) can colonize or disseminate in water, mud and soil (Asi et al., 2020). The clear spatial relationship we have found between environmental contamination and co-infection of residents at the neighborhood level suggests that this explicit spatial environmental assessment approach could provide an important shortcut in fieldwork to then more effectively address public issues. Soil contamination of other public spaces, such as parks, public squares and beaches, recorded in other studies, is an important means of transmission of zoonoses (Ribeiro et al., 2013; Silva et al., 2017

V.CONCLUSION

Our study of intestinal parasites in human and environmental samples carried out in the Commune of Yaoundé IV allowed us to isolate and identify the eggs and larvae of enteropathogenic helminths such as *Ascaris lumbricoides, Strongyloides stercoralis, Ankylostoma duodenale* etc. The results showed the presence of the parasitic cycle of strongweed in the studied area due to excellent soil conditions, revealing the importance of adopting prophylactic measures to ensure the health of each individual, based on the care of the soil.

The proximity of homes and their contaminated environment contributes to the spread of progressive parasitic forms and reinforces the epidemiological triad of infection. The ability to combine environmental and human field investigations to identify key components acting at different levels improves the potential for using new knowledge and tools to combat neglected tropical diseases. In order to limit the health risks linked to soil contamination and avoid a major transmission of these groups of organisms, we can recommend an application of hygiene at all levels, nutrition, environment, body and habitat.

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