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Prevalence of Soil-transmitted Helminths and Intestinal Protozoa in Shanty Towns of Libreville, Gabon

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Authors' contributions

This work was carried out in collaboration between all authors. Author PNM collected the samples and performed the technical work. Author FMM participated to the technical work and data collection. Authors MKBA and PDMM designed the study and wrote the protocol. Author MK solved the technical problems. Authors PNM and MKBA did the statistical analysis, wrote and corrected this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Intestinal parasitic infections (IPIs) are significant public health problems in sub-Saharan Africa. Establishment of effective control strategies such as mass drug administration of anthelminthic are implementing while recent local data in urban areas are lacking.
Aim: To assess the frequency of IPIs and coparasitism in patients from shanty towns of Libreville, the capital city of Gabon.
Study Design: Cross-sectional and observational study.
Place and Duration of Study: Department of Parasitology Mycology at the Université des Sciences de la Santé, Libreville, from February to April 2014.

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Methods: Stool samples collected from 101 individuals, aged between 13 and 74 years old, were analysed by direct microscopic examination, Merthiolate-Iodine-Formaldehyde concentration and coproculture. Age, education level, type of house and existence of latrine were also recorded.
Results: Among analysed stools, 75.2% (n=76/101) were infected with at least one parasite. Protozoa represented 94.3% of the detected parasites with *Blastocystis hominis* being the predominant species (41.6%; n=42/101). *Entamoeba histolytica/dispar* (6.9%; n=7/101)) and *Giardia intestinalis* (2.0%; n=2/101) were the less common protozoa. Soil-transmitted helminths (STH) were found in 7.9% (n=8/101) of stool examined, *Trichuris trichiura* (5.9%) was the most common helminth detected, followed by *Ascaris lumbricoides* (2.0%), hookworm (1.0%) and *Strongyloides stercoralis* (1.0%). Coparasitism was present in 35.6% (n=36/101) of positive cases. Dual and triple infections accounted for 23.8% and 6.9%, respectively. Having no education and living in a wooden house were associated with IPIs ($p<0.01$), particularly STH which were more frequent among women and children (p<0.01).
Conclusion: This study reports a high frequency of intestinal parasites infection in shanty towns of Libreville. An unexpected higher prevalence of protozoa compared to STH is also noticed.

Keywords: Gabon; IPI; shanty town; polyparasitism; risk factors.

1. INTRODUCTION

Intestinal parasitic infections (IPIs) due to soil-transmitted helminth (STH) and protozoa infections have been recognized as neglected tropical diseases (NTDs) and as one of the most significant causes of illnesses among disadvantaged communities from SubSaharan Africa [1,2]. In 2010, it was estimated that IPIs result in 400 to 800 million illness cases [3]. These infections are ubiquitous with high prevalence among the poor and socioeconomically deprived communities who undergo overcrowding, poor environmental sanitation, low level of education and lack of access to safe water, warm climate, lack of personal or environmental hygiene, and poor health or nutritional status [4]. These factors increase the risk of being contaminated, trapping exposed populations in a perennial cycle of poverty [1,4]. IPIs identification is important, since some are due to pathogenic organism and, others considered up to now as non-pathogenic, seem to induce intestinal and allergic symptoms that can be chronic. Indeed, STH are recognized as pathogens inducing clinical symptoms while the pathogenic role of protozoan, other than *Entamoeba histolytica* and *Giardia intestinalis* such as *Blastocystis hominis*, remains either controversial or less investigated [5]. Previous studies reported that *B. hominis* is probably the most common human gut protozoan in the world, with >50% of prevalence in developing countries but the relationship between its presence and the clinical digestive symptoms is controversial [6,5].

Urban migration leads to the creation of precarious urban settlements, with a growth process of shantytowns characterized by poor housing conditions, narrow streets that are not paved, a lack of sanitation, clean water supply and electricity, as well as a poor accessibility to health services. This phenomenon occurs in Libreville, the capital city of Gabon where more than 60% of the total population live, with a high proportion located in shanty towns. Data on intestinal parasitism are scarce in this city. Those available focus on STH and were recorded more than 8 years ago in rural areas while since several years, access to safe water is difficult due to frequent supply interruptions. People are supplied in common water sources, such as pumps and then transport the collected water to their home and store it in tanks. Thus, the risk of human and/or waterborne transmission of IPIs would be actually high. Moreover, national deworming programmes are being prepared by national control programs for country implementation. They consist of mass administration of albendazole and praziquantel highly effective against STH whom prevalence estimates remain unknown. It is therefore essential to identify the real burden and reservoirs of these parasites, to facilitate the establishment of effective control strategies. Furthermore, while these programs focus on preschool-aged and school-aged children from semi-urban and rural areas, important gaps regarding IPIs among adults exist. During the weekly consultation performed at the Department of Parasitology Mycology (DPM), patients with symptoms such as pruritus and or abdominal pain are routinely screened for blood and IPIs. The present study was designed to estimate the frequency of IPIs in patients from shanty towns of

Libreville consulting at the DPM during a 4 months period.

2. PATIENTS AND METHODS

2.1 Study Site

This was a cross-sectional and observational study performed at the Department of Parasitology Mycology (DPM) of the Faculty of Medicine of Libreville, from February to April 2014. The study was conducted in Libreville, the capital city of Gabon, in Central Equatorial Africa. Libreville is located on the northwest coast and is built on rugged ground, with numerous hills and valleys crossed by streams more or less important that flow into the estuary. The population of Libreville was estimated at 850,000 inhabitants in 2014, representing almost 54% of the total population. Urban density is high, with 250 to 300 inhabitants per km^2, and urbanization is anarchic and uncontrolled. Thus, shanty towns occupy almost two third of the urban space in Libreville; the 3rd and the 6th districts are shanty towns with approximately 150 000 inhabitants who live in precarious conditions. In Gabon, the climate is equatorial, characterized by an alternation of two dry (December– January and June–September) and two rainy (February– May and September–December) seasons. The average annual rainfall is about 1,600–1,800 mm. The mean temperature is about 24℃.

2.2 Study Population and Procedures

Patients seen during medical consultations performed at the DPM of the Faculty of Medicine were invited to participate to the study. Among the forty to sixty patients seen weekly, approximately one half undergo a biological testing due to the presence of clinical signs suggestive of parasitic infection, or as an additional test for a global check-up. Individuals with a negative diagnosis of filariasis and those presenting symptoms likely associated with IPIs such as pruritus or urticaria, digestive symptoms or a combination of both signs, usually benefit from stool examination. In the present study, patients were included if they met the following criteria :to live in the 3rd or the 6th district of Libreville, to provide a stool sample, not leaving their place of residence for at least six months, to have available parasitological test results, to have given their oral informed consent for data use. The demographic, socioeconomic and sanitary information as well as symptoms were collected by trained field researchers, through semi-structured questionnaires. The interviewer wrote down the answers and the questionnaire was checked before releasing the patient. In case of illiteracy, interview was performed in local language with the help of the person accompanying the patient for the consultation. All collected stool samples were macroscopically examined, then prospectively analysed by routinely used standard methods: direct microscopic examination of a small amount of stool mixed in a saline solution drop (0.9% sodium chlorure), or fixed stools with 10% formaldehyde, and after the merthiolate-iodine-formaldehyde coloration and concentration (MIFc) of eggs, cysts and vegetative forms according to Sapero and Lawless [7]. MIFc is a simple and faster colouring method which consists of concentrating approximately 1 gram of fecal sample crashed in the test solution and stained with 5% lugol solution. The combination of MIF and lugol for the coloration has a good sensitivity for the identification, the fixation and the conservation of vegetative forms and cyst of protozoa. Iodine and eosin facilitate the recognition and differentiation by well-trained technician of the nuclear membrane and other cytoplasmic inclusion which are specific for each species. Species identification is carried out as follows. First, the marked cyst or vegetative form is detected at magnification x10 or x40; after perfectly centred and an addition of a drop of immersion oil on the slide, the parasite is recognized at magnification x 100. On a bright background (light pink cytoplasm), cysts stand clear, the nuclei appear as clearly drawn in pink or red orange, the nuclear membrane appears in dark brown, chromatin appears darker. The siderophilic bodies remain colourless and refracting and vacuoles are mahogany. Red cells appear bright red [8].

Parasite coproculture based on the Harada and Mori technique was performed for the detection of helminth larvae [9]. Two trained laboratory technicians undertook the examination using optical microscopes. The result was considered positive if eggs or larvae (for helminths) or cysts/vegetative forms (for protozoa) were found by at least one method. They were considered negative if the entire slide or pellet did not reveal any parasite.

2.3 Ethical Considerations

All tests performed were part of the routine diagnosis of IPIs in the DPM. Oral informed consent for the use of data was obtained from each participant after the study had been explained. An oral assent was sought from children aged between 12 and 18 years with the oral consent of their parents. Results were reported within three days and positive patients were seen by the physician and treated according to the national recommendation. Data analysis was performed in agreement with the Gabonese Ministry of Health.

2.4 Statistical Analysis

Data collected were entered into an Excel spreadsheet and analysed using the Stata® version 12.1 software (StataCorp LP, Texas, USA). The statistical tests performed included the Pearson's Chi-square and Fischer's exact for group comparisons. Statistical significance was set at $p < 0.05$.

3. RESULTS

Data from 101 individuals with a median age of 40[24-54] years were analysed. Their characteristics are presented in Table 1. Almost half (n=44, 43.6%) had a secondary school level, 35 (34.7%) lived in houses made of wood or sheet metal and 53 (52.5%) used latrines. Overall, 26 patients presented digestive symptoms, as single symptoms in 10 patients (9.9%) and associated with pruritus in 16 patients with abdominal pain (n=13) or with diarrhoea (n=3).

IPIs were detected in 76 (75.2%) stool samples with a predominance of protozoa among infected ones (n=76/76; 100.0%) (Table 2). Helminth infections were always associated with protozoa species in positive samples (Table 2 and 3). The most commonly found parasite was *Blastocystis hominis* followed by *Entamoeba coli* and *Endolimax nana* (Table 2); 4 diarrheic out of the 7 stools with *Entamoeba histolytica/dispar* carried both vegetative forms and cysts. *Trichuris trichiura* (5.9%) was the more frequently detected STH species while larva of hookworm and *Strongyloides stercoralis* were rarely present (Table 2). Patients with polyparasitism carried two to five different species (Table 1). The rate of infected samples with pathogenic protozoa including *B. hominis* was higher than that of non-pathogenic (n=42/75; 56.0% *versus* n=25/75; 33.3%; $p<0.01$). Cysts of *Iodamoeba butschlii* were not detected. One sample infected with *E. histolytica/dispar* had also vegetative forms of *Chilomastix mesnili*. Among the five samples infected by cysts or vegetative forms of *Entamoeba hartmanni*, three also had *B. hominis* and *E. coli*, and two *E. histolytica/dispar* and other parasites. *B. hominis* was the only parasite found in samples from 11 patients with digestive symptoms.

Table 1. Characteristics of the study population

Characteristics	**Median**	**25^{th}–75^{th} quartile**
Age	**40**	**24-54**
	N	**%**
Adults	90	89.1
Female	66	65.3
Type of house		
Wooden house	24	23.8
Brick house	66	65.3
Metal sheet house	11	10.9
Educational level		
None	11	10.9
Primary school	33	32.7
Secondary school	48	47.5
High school	9	8.9
Type of toilets		
Interne toilets	48	47.5
Latrines	53	52.5
Reported dewormed the last six months	15	14.8
Symptoms		
Abdominal pain	23	22.8
Diarrhoea	4	4.0
Pruritus or urticaria	91	90.1

The prevalence of IPIs was significantly higher among individuals who live in a wooden house and among those who had no education ($p<0.01$) (Table 4). Coparasitism rate (27.8%; n=25/90) was almost twofold lower among adults compared to children (54.5%; n=6/11) but this difference was not statistically significant ($p=0.14$). Pathogenic species were significantly more prevalent in case of illiteracy (75.0% *versus* 29.8%; $p<0.01$). STHs were more frequently found among children ($p<0.01$) and women ($p<0.01$) (Table 4). The same trend was observed in the group of patients who live in a wooden house ($p=0.06$) as well as in the group of illiterate ($p=0.09$), while no difference was found according to the type of toilets (Table 4). No socio-demographic characteristic-related differences were found for intestinal protozoa infection.

4. DISCUSSION

IPIs mapping is essential for the design or the improvement of MDA strategies. National deworming programmes were recently included in the package of strategies for the control of endemic parasitic neglected diseases in Gabon. Thus, data should be generated and available for the national control program. However, accurate data are scarce in the country, where only those from rural areas or very old ones are available [10,11].

The present cross-sectional study determining the prevalence of soil transmitted helminths and intestinal protozoa among 101 individuals revealed that intestinal parasitic infections are frequent in shanty towns of the urban city of Libreville. The purpose of this work was not to induce a decision at country level by policy makers. It was designed as a pilot study performed in an urban city and among adults. Indeed, they represented the majority of the included individuals; additionally, 75 out of the 101 patients did not present any digestive symptoms. Thus, as demonstrated elsewhere, they could represent a subset of the parasite reservoir living in shanty towns, providing important information for public health [12,13]. Moreover, weekly observations generated by the results of the lab diagnosis showed a low frequency of STH which are targeted by deworming programs. The present work, although obtained in a small sample size of symptomatic patients highlights the predominance of protozoan parasites. Further studies in urban and rural areas with higher sample size and in all age populations would confirm the present findings.

The prevalence of IPIs was 75.2%, similar to rates reported more than 20 years ago in Libreville (76%), in an eastern rural area (100%) as well as recently found in a population of Tanzanian adults (83%) [14-16]. Such prevalence are higher than those found at Lambaréné, a rural city located almost 300 km from Libreville (49%) and in other countries such as Ethiopia (45.3), Cameroun (44.1%) and Rwanda (50%), this infection rate is higher. [17-20]. This difference is likely due to the concomitant detection of helminths and protozoa by the MIF concentration. Indeed, studies previously performed in the country focused on helminth species and Kato-Katz was used for parasite detection. Moreover, study populations were children and pregnant women who are more often infected by STH [14,17,21,22].

Commensal enteroparasites warrant a specific attention because their transmission pathways can be identified by behaviours patterns and other conditions that contribute to the spread of other diseases via the same route of transmission [1,23]. Indeed, knowledge of transmission patterns and the prevalence of intestinal parasitism are useful when implementing preventive strategies at a public health level. Prevalence rates of *E. coli, E. nana, E. hartmanni* are considerable, underlining the poor living conditions of inhabitants from these areas.

The predominance of protozoa, more precisely of pathogenic ones, is in agreement with data from Ethiopia (70.8% *versus* 5.2%) and Rwanda (54.5% *versus* 20), but in contrast with others [2,21,24,25]. Surveys performed 30 years ago in Gabon, showed prevalence of STH infections of 75% or higher [14,16]. Frequent self-medication with antihelminthic drugs as well as deterioration of populations living conditions could partly explain the observed profile of intestinal parasitism. Unplanned urban expansion with environmental components such as water, food, cooking facilities, housing, and health-care are also risk factors for IPIs. With the frequent water supply shortage, common sources of water were created, increasing the risk of contamination during transporting and storing of drinking water supply between the source and the point of use, as demonstrated elsewhere [26,27].

Otherwise, intestinal protozoa such as *E. histolytica* and *G. intestinalis* have clinical importance and impact on the quality of life of infected individuals. Their frequency is relatively low, in accordance with data obtained in other areas of the country and outside Africa. However, 4 out the 7 stools infected with *E. histolytica* contained both cysts and vegetative forms. Considering that molecular analysis often discriminate *E. histolytica* among collected cysts, actions against this pathogenic amoeba should be envisaged for therapeutic or preventive management [28]. Contamination of drinking water with *Giardia* cysts can occur during transporting and storing of water as population uses containers to transfer water from the dams or wells to the houses where water is stored to be used. Moreover, faecal contamination of water between the source and the house is reported [27].

B. hominis was the most predominant pathogen species. It was found as a mono-infection in 11 out of the 26 symptomatic patients. Its pathogenic role, although controversial, is being highlighted by several authors. Its association with clinical sign such as urticarial, abdominal pain, irritable bowel syndrome and diarrhoea in the absence of another aetiology was already demonstrated [29-30]. Screening of *B.hominis* should be envisaged in symptomatic patients, as well as during epidemiological studies.

Compared to *A. lumbricoides* and hookworm, *T. trichiura* was the most frequently detected STH. Its prevalence is high with an estimation 464.6 million people infected worldwide, mainly in sub-Saharan Africa and Asia [1]. A possible explanation is the common self-medication practice with albendazole or mebendazole in Libreville, both drugs do not have 100% efficacy against *T. trichiura* [19,20,31,32].

Our study has some limitations. The sample size was small and only one stool sample was collected from each participant. It was hospital-based, not performed at a community level. Furthermore, the intensity of IPIs was not determined because the Kato-Katz technique was not used. However, MIF coloration and concentration have a good sensitivity for common STH and protozoan species detection, including schistosomiasis, although kato-katz remains the reference method for *Schistosoma mansoni* detection and quantification. This species as well as *Taenia spp* eggs are not endemic in Gabon. Additionally, in the DPM, patients are treated based on the presence of parasite whatever the parasite density. Finally, molecular analysis which could diffenciate *E. histolytica* from *E. dispar*, as well as *Blastocystis hominis* strains, was not performed.

Although this study was not aimed at analysing epidemiological factors associated with IPIs carriage, and the small sample size which probably underpowered the statistical analysis, some factors were found associated either with the high prevalence of IPIs (living in a wooden house and being illiterate) or with STH carriage (age, sex, type of house) in agreement with reports from others [6,18,21]. Other factors such as poor individual hygiene, bad eating habits, low socioeconomic status, poor living conditions, insufficient knowledge and practice of correct sanitation could also be involved.

Table 2. Profile of intestinal parasitism

Parasite carriage	N	%
Helminths	8	7.9
Protozoa	76	75.2
Monoparasitism	40	39.6
Coparasitism	36	35.6
Protozoa + helminths	8	7.9
2 parasites	24	23.8
3 parasites	7	6.9
4 parasites	4	4.0
5 parasites	1	1.0

Table 3. Prevalence of parasite species detected in the study population

Species	Total		Monoparasitism		Coparasitism	
	N	%	N	%	N	%
Helminths						
Ascaris lumbricoides	2	2.0	0	0.0	2	100.0
Hookworm	1	1.0	0	0.0	1	100.0
Strongyloides stercoralis	1	1.0	0	0.0	1	100.0
Trichuris trichiura	6	5.9	0	0.0	6	100.0
Protozoa						
Blastocystis hominis	42	41.6	17	40.5	25	59.5
Endolimax nana	28	27.7	8	28.5	20	71.4
Entamoaba coli	33	32.7	12	36.4	21	63.6
Entamoeba hartmanni	7	6.9	1	14.3	6	85.7
Entamoeba histolytica/dispar	7	6.9	1	14.3	6	85.7
Giardia intestinalis	2	2.0	1	50.0	1	50.0

Table 4. Frequency of IPIs according to socio-demographic data

Characteristics	Infected		Non infected		STH		Pathogenic protozoa	
	N	%	N	%	N	%	N	%
Age								
Adults	67	64.4	23	25.6	6	6.7	36	40.0
Children	9	82.8	2	18.2	2	18.2*	6	54.4
Sex								
Female	48	72.7	18	27.3	6	9.1*	24	36.4
Male	28	80.0	7	20.0	2	5.7	18	51.4
Type of house								
Wooden house	24	100.0*	0	0.0	4	16.7	12	50.0
Brick house	43	65.1	23	34.9	3	4.5	23	31.8
Metal sheet house	9	81.2	2	10.8	1	9.1	7	63.6
Educational level								
None	11	100.0	0	0.0	2	18.2	9	81.8
Primary school	23	69.7	10	30.3	4	9.3	18	54.5
Secondary school	37	77.1	11	22.9	2	4.2	26	68.4
High school	7	77.8	2	22.2	0	0.0	5	55.6
Type of toilets								
Interne toilets	38	79.2	10	20.8	2	4.2	20	62.5
Latrines	38	71.7	15	28.3	6	11.3	29	54.7

**: difference statistically significant*

5. CONCLUSION

Intestinal parasitic infections are highly prevalent and should be considered as major public health concerns among the poor and socioeconomically communities living in some shanty towns in the capital city of Libreville, Gabon. Further studies should be performed to confirm the predominance of protozoa infections among IPIs and to identify IPI transmission mechanisms in order to design appropriate control strategies. If the low frequency of STH is confirmed, the design of actual deworming programmes which are based on antihelminthic mass administration should be revised.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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