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Prevalence and Sociodemographic Factors Associated With Chronic Intestinal Polyparasitism in Non-Pregnant Adults Living In Urban and Rural Areas of Gabon

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ABSTRACT

Introduction: *Intestinal parasitic infections constitute a major public health problem worldwide. Data from non-pregnant adults are scarce in high burden countries such as Gabon. The present study aimed to determine the prevalence and features of intestinal polyparasitism in urban and rural areas of Gabon.*

Methods: *Apparently healthy volunteers with a microscopic diagnosis of intestinal parasites were included. Sociodemographic and lifestyle characteristics were collected using a written questionnaire. The type and number of detected parasites were also recorded. Statistical analysis was performed using SPSS version 20.0.*

Results: *Among the 239 participants, the prevalence of polyparasitism was 36.4%. The frequencies of protozoa, soil-transmitted helminths (STHs) infections and co-infection (protozoa-STHs) were 35.6%, 46.4% and 18.0%, respectively. Univariate and multivariate analysis showed that polyparasitism was associated with living in rural area (aOR=3.6; 95%CI = 1.0 - 12.1, p = 0.03), female gender (OR = 1.8; 95%CI = 1.0 - 3.2, p = 0.025), the use of non-conventional latrines (aOR = 3.3; 95%CI =1.2 - 9.3, p = 0.02). Drinking river water (aOR = 2.6; 95%CI = 1.1- 6.0, p = 0.024) and living in urban area (uOR = 2.9; 95%CI =1.3 - 6.6, p = 0.007) were significant risk factors of STHs and protozoa carriage, respectively.*

Conclusion: *Polyparasitism is frequent in adults. It is associated with poor sanitary conditions in rural sites. Awareness campaigns and integrated control strategies should also target adult population, which constitutes a significant parasite reservoir.*

Keyword

Intestinal parasites, Polyparasitism, Risk factors, Gabon.

Introduction

Intestinal parasitic infections (IPIs) are among the most widespread neglected tropical diseases in the world [1,2]. These infections are predominant in developing countries and are mainly caused by two parasitic groups: protozoa and helminths [2,3]. Infections due to pathogenic intestinal protozoa such as *Giardia duodenalis (G. duodenalis)* and *Entamoeba histolytica* (*E. histolytica)* are the leading cause of morbidity [4]. STHs notably *Ascaris lumbricoides (A. lumbricoides), Trichuris trichiura* (*T. trichiura)*

and hookworms affect 24% of the world's population [5]. Poverty, inadequate sanitation facilities, improper waste disposal systems, lack of drinking water supply and low economic status are known factors associated with IPIs [3,6,7]. IPIs may be due to the presence of one or more parasite species within the organism leading to monoparasitism and polyparasitism, respectively. In endemic areas, chronic parasitism, a consequence of IPIs, is common. Untreated asymptomatic chronic IPIs carriers may be more frequently infected by multiple parasites species. Burden of chronic carriage of multiple parasites is under-estimated although it may result on greater impact on morbidity compared to single parasitism [8-10]. Moreover, the risk factors associated with polyparasitism are not well described. Indeed, polyparasitism could increase the severity of certain diseases, as well as children susceptibility to other infections, malnutrition and growth disorders [11-13]. On the other hand, the multiple intestinal parasitic species could synergistically disrupt intestinal homeostasis causing dysbiosis with digestive or extra-intestinal impact [14,15]. While some studies highlight the non-negligible frequency of IPIs in adults living in tropical areas, data on polyparasitism in adults are scarce [16,17]. They are not targeted by preventive strategies such as mass drug administration. Thus, they should constitute a significant parasite reservoir, able to maintain household contamination of children who are more susceptible to IPIs consequences (like intestinal bleeding, anemia, malnutrition, mental and physical development retardation [18,19]. Such information's are important for the design of various control strategies. In Gabon, IPIs are widespread in both urban and rural areas [20-22]. The burden and risk factors of intestinal polyparasitism has been less investigated in non-pregnant adults. Therefore, the aim of this study was to investigate the epidemiology of chronic intestinal polyparasitism among apparently healthy individuals living in rural and urban areas of Gabon. This information will contribute to the development of appropriate integrated control strategies for the reduction of the transmission of IPIs.

Materials and Methods

Study type and areas

This was a cross-sectional study carried out from September 2020 to November 2021 in three settlements in Gabon, including Libreville (an urban area), as well as Koula-Moutou and Bitam (both classified as rural areas). Libreville, the capital of Gabon, with approximately 703,939 residents. Situated in the southeastern region of Gabon, Koula-Moutou serves as the principal city of the Ogooué-lolo province, located 588 km away from Libreville, and where 25,651 inhabitants live. Bitam is situated in the northern region of Gabon, specifically in the province of Woleu-Ntem. Approximately 111,923 people live at Bitam. The recruitment process was meticulously carried out by the trained teams of the Centre de Recherche en Pathogènes Infectieux et Pathologies Associées (CREIPA) in Melen, in the Centre Hospitalier Régional Paul Moukambi of Koula-Moutou (CHRPM) and at the medical center of Bifolossi in Bitam.

Study Population and Sample Size Calculation

This study was nested in a survey performed in Gabon (Parcam project), in which apparently healthy adults with the following criteria were included: age over 17 years old, absence of chronic infection (as determined by HIV, malaria, hepatitis, tuberculosis testing, absence of digestive symptoms the last six months, absence of self-medication with an anthelminthic or metronidazole the last six months, absence of known chronic and/or severe disease) permanent presence in the study areas the last two years and signed informed consent.

The sample size was calculated with the formula used to estimate a sample proportion: $(n=z^2p(1-p)/e^2)$, the sample size for this study was estimated considering a prevalence of polyparasitism of 17.3% [22], a 5% level of significance and 95% confidence interval (CI). Therefore, a minimum of 219 participants was required.

Questionnaire survey

Data were collected using a survey questionnaire designed by the study team. Demographics factors (age, gender, level of school attendance) and living conditions (i.e., wear shoes outdoors, type of toilets, drink water sources, presence of a watercourse close to the home) were recorded.

Fecal sample collection and testing

After interview and physical examination, labelled stool vials and spatulas were given to participants for fresh stool collection the next morning. They were instructed on the proper method of stool collection in order to avoid contamination. The samples were transported the morning of the day of collection, for laboratory testing. Direct microscopic examination, merthiolate-iodine-formaldehyde (MIF) coloration and concentration techniques, coproculture were performed for all the samples. They were considered as positive (IPIs) if at least one egg and/or one cyst and/or one larva and/or one vegetative form were detected with at least one of these four microscopic technics.

Ethics consideration

Authorization for the study realization was sought and obtained from the Ministry of Public Health of the Gabonese Republic. The protocol was approved by the National Ethics Committee (under reference PROT N°002/2020/PR/SG/CNE). The infected patients received appropriate antihelminthic or antiprotozoal treatment according to the national guidelines.

Statistical analysis

Statistical analysis was performed using STAT View and SPSS version 20.0. The data analysis strategy focused on the description of the study population (univariate analysis), the crossing of variables (bivariate analysis). Shapiro's test was used to check whether quantitative variables followed a normal distribution. Means (±SD) and median [IQR] were represented for quantitative variables. Proportions were compared using Chi-square and Fisher test as appropriate. Multivariate logistic regressions and multinominal logistic regression were carried out to assess the relationship between each variable (monoparasitism and polyparasitism) by adjusting for potential confounding factors (age, sex, the study area, school attendances and occupation), unadjusted (uOR) and

adjusted (aOR) are presented with 95% confidence intervals (CI). The p-value values <0.05 were considered significant.

Results

The main study included 997 volunteers, 347 (34.8%) were found positive but only 239 of the volunteers benefited from all the parasitological tests and were interviewed. Among the 239 individuals with IPIs, the majority lived in rural settings (Figure 1).

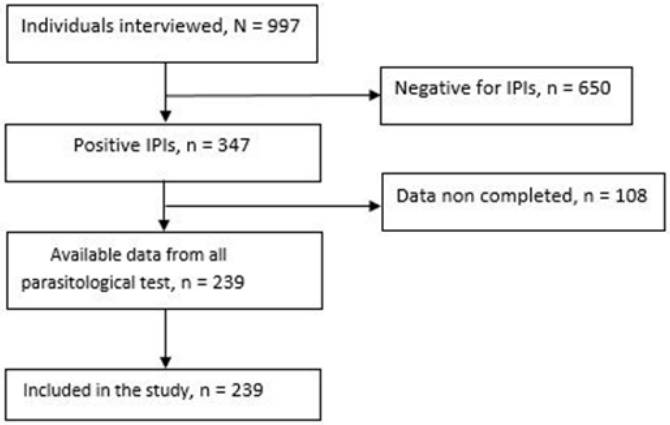

Figure 1: Study flow chart.

The median age was 41 [31-51] years in the study population. A proportion of 79.6% (n = 189) of the participants had over 29 years old. The majority of the volunteers (85.3%; n = 204) had a primary or secondary school level, were active (69.6%; n = 165), drank river or mixed water (73.2%; n = 175) and used latrines (86.6%; n = 207) (Table 1).

Table 1: General characteristics of the study population.

Characteristics		**N**	**(%)**
Areas of residences	Urban	32	13.4
	Rural	207	86.6
Gender	Male	108	45.2
	Female	131	54.8
Age groups (years)	< 30	50	21.0
	30 - 59	178	75.0
	≥ 60	11	4.6
School attendance	None	17	7.1
	Primary	109	45.4
	Secondary	95	39.6
	University	18	7.5
Occupation	Unworkers	52	22.0
	Students	18	7.6
	Self-employed	112	47.3
	Workers	53	22.3
	Retired	2	0.8
Sources of drinking water	River	149	62.3
	Tap water	64	26.8
	Mixed	26	10.9
Types of toilets	Non-conventional latrines	167	69.9
	Conventional latrines	32	13.4
	Modern	40	16.7
Wearing shoes when outside regularly		179	74.9
Presence of a stream near the house		58	24.3

The prevalence of protozoan and STHs were 53.6% and 64.4%, respectively. Seven parasites species were identified: *T. trichiura* (39.7%), *Blastocystis sp* (38.0%), *A. lumbricoides* (24.7%), *E. hystolytica/dispar* (24.2%), *Necator americanus* (11.3%), *G. duodenalis* (3.34%) and *Strongyloides stercoralis* (3.34%).

The average number of parasites per participant was 1.44 ± 0.6. The frequency of polyparasitism was 36.4% (n = 87/239) (Table 2). It was more frequent in rural compared to urban areas (40.1% vs 12.5%; aOR = 3.6; 95%CI = 1.0 - 12.1, $p = 0.038$) (Table 2). Being women (uOR = 1.8; 95%CI = 1.0 - 3.2, $p = 0.025$) and using non-conventional latrines (aOR =3.3; 95%CI =1.2 - 9.3, $p = 0.02$) were associated factors with polyparasitism. After adjustment, the association of polyparasitism with female gender was not significant. People living in urban areas were three times at risk to have monoparasitism compared with those from rural settings (87.5% vs 59.9%; aOR = 3.4; 95%CI = 1.0 - 11.6), $p = 0.044$) (Table 2).

STHs, protozoan and STHs/Protozoan IPIs were detected in 46.4%, 35.6% and 18.0% of the participants, respectively. The mean number of protozoan and geohelminths species was the same 1.22 (±0.46) parasites. In rural areas, 48.3% of individuals were infected by STHs only. Being self-employed (aOR = 3.2; 95%CI = 1.4 -7.34, $p = 0.006$), drinking river water (aOR = 2.6; 95%CI = 1.1 - 6.0, $p = 0.024$) and using non-conventional latrines (aOR = 5.6; 95%CI = 2.1 - 14.9, $p = 0.001$) were risk factors for STHs carriage (Table 3). Being infected by both helminths and protozoa was more common in rural areas (20.8%), as well as among participants who drank water of mixed origin (27.0%) (Table 3).

The carriage of protozoa was associated with living in urban area (uOR = 2.9; 95%CI = 1.3 - 6.6, $p = 0.007$), being a student (uOR = 3.4; 95%CI = 1.1 - 10.1, $p = 0.027$), drinking tap water (aOR = 2.6; 95%CI = 1.0 - 6.4, p = 0.036) and using modern toilets (aOR = 11.9; 95%CI = 2.9 - 48.3, $p = 0.001$) (Table 3). After adjustment, the association of protozoa carriage with urban site and school attendance was no longer significant.

Globally, the participants from the urban sites carried more frequently one or two parasites. A single STH specie carriage was found in 36.0% (n = 86) of participants while a single protozoan specie carriage concerned 66 (27.6%) participants. Two volunteers

Table 2: Relationship between sociodemographic factors and mono or polyparasitism.

Characteristics		Monoparasitism (N=152)	Polyparasitism (N=87)	*P-value*
Areas of residence, n (%)	Urban	28 (87.5)	4 (12.5)	**0.004**
	Rural	124 (59.9)	83 (40.1)	
Gender, n (%)	Male	77 (71.3)	31 (28.7)	**0.034**
	Female	75 (57.3)	56 (42.7)	
Age groups (years), n (%)	< 30	28 (56.0)	22 (44.0)	0.450
	30 – 59	117 (65.7)	61 (34.3)	
	≥ 60	7 (63.6)	4 (36.4)	
School attendance, n (%)	None	10 (58.8)	7 (41.2)	0.118
	Primary	65 (59.6)	44 (40.4)	
	Secondary	60 (63.8)	34 (26.2)	
	University	16 (88.9)	2 (11.1)	
Occupation, n (%)	outworkers	27 (52.0)	25 (48.0)	**0.054**
	Students	12 (66.7)	6 (33.3)	
	Self-employed	68 (60.7)	44 (39.3)	
	Workers	42 (79.3)	11 (20.7)	
	Retired	1 (50.0)	1 (50.0)	
Sources of drinking water, n (%)	Natural sources	94 (63.1)	55 (36.9)	0.682
	Tap water	43 (67.2)	21 (32.8)	
	Mixed	15 (57.7)	11 (42.3)	
Type of toilets, n (%)	Non-conventional latrines	95 (56.9)	72 (43.1)	**0.002**
	Conventional latrines	23 (71.9)	9 (28.1)	
	Modern	34 (85.0)	6 (15.0)	
Wearing shoes when outside regularly, n (%)	Yes	116 (64.8)	63 (35.2)	0.606
	No	36 (60.0)	24 (40.0)	
Presence of a stream near the house, n (%)	Yes	37 (63.8)	21 (36.2)	1.00
	No	115 (63.5)	66 (36.5)	

Table 3: Factors associated with the type of parasitism.

Characteristics		STHs (N=111)	STHs-Protozoa (N=43)	Protozoa (N=85)	*P-value*
Areas, n (%)	Urban	11 (34.4)	0 (0.0)	21 (65.6)	**< 0.001**
	Rural	100 (48.3)	43 (20.8)	64 (30.9)	
Gender, n (%)	Male	54 (50.0)	17 (15.8)	37 (34.2)	0.552
	Female	57 (43.5)	26 (19.9)	48 (36.6)	
Age groups (years), n (%)	< 30	24 (48.0)	6 (12.0)	20 (40.0)	0.380
	30 - 59	82 (46.1)	33 (18.5)	63 (35.4)	
	≥ 60	5 (45.5)	4 (36.4)	2 (18.1)	
School attendance, n (%)	None	6 (35.3)	2 (11.7)	9 (53.0)	0.089
	Primary	56 (51.4)	23 (21.1)	30 (27.5)	
	Secondary	43 (45.7)	17 (18.0)	34 (36.3)	
	University	6 (33.3)	1 (5.6)	11 (61.1)	
Occupation, n (%)	outworkers	19 (36.5)	13 (25.0)	20 (38.5)	**< 0.001**
	Students	4 (22.2)	1 (5.6)	13 (72.2)	
	Self-employed	65 (58.0)	23 (20.5)	24 (21.5)	
	Workers	22 (41.5)	5 (9.5)	26 (49.0)	
	Retired	0 (0.0)	1 (50.0)	1 (50.0)	

Sources of drinking water, n (%)	River	83 (55.7)	27 (18.1)	39 (26.2)	**< 0.001**
	Tap water	18 (28.2)	9 (14.0)	37 (57.8)	
	Mixed	10 (38.4)	7 (27.0)	9 (34.6)	
Type of toilets, n (%)	Non-conventional latrines	87 (52.1)	35 (20.9)	45 (27.0)	**< 0.001**
	Conventional latrines	16 (50)	6 (18.7)	10 (31.3)	
	Modern	8 (20.0)	2 (5.0)	30 (75.0)	
Wearing shoes when outside regularly	Yes	84 (46.9)	32 (17.9)	63 (35.2)	0.966
	No	27 (45.0)	11 (18.3)	22 (36.7)	
Presence of a stream near the house, n (%)	Yes	19 (32.7)	13 (22.5)	26 (44.8)	**0.052**
	No	92 (50.8)	30 (16.6)	59 (32.6)	

Table 4: Distribution of the number of parasites according to the type of parasitism in urban and rural areas.

Variables, n (%)	0 Helminth		1 Helminth		2 Helminths		3 Helminths	
	Urban	Rural	Urban	Rural	Urban	Rural	Urban	Rural
0 Protozoa	0 (0.0)	0 (0.0)	10 (90.9)	76 (76.0)	1 (9.0)	21 (21.0)	0 (0.0)	3 (3.0)
1 Protozoa	18 (100.0)	48 (57.8)	0 (0.0)	29 (34.9)	0 (0.0)	6 (7.2)	0 (0.0)	0 (0.0)
2 Protozoa	3 (100.0.0)	14 (63.6)	0 (0.0)	8 (36.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
3 Protozoa	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

(0.8%) were infected by 3 protozoan, and 3 (1.3%) by three different STH species , all lived in rural areas (Table 4). Protozoa-STHs carriage with at least three different species was observed in 14 (5.9%) inhabitants from the rural settlements: eight (8) carried one (1) STH and two (2) protozoa, and six (6) had two (2) STHs and one (1) protozoa.

Discussion

IPIs continue to pose a significant health challenge, as evidenced by the high prevalence observed in endemic regions worldwide [2,23,24]. In Gabon, some epidemiological studies have reported the extent of intestinal polyparasitism [20-22]. Investigating the factors associated with multiple parasitic infections, as well as differences between urban and rural areas, could provide valuable insights for the control of IPIs. As previously reported in children and adults, STHs and protozoa are common (64.4% and 53.6% respectively) [22,25]. In urban cities and rural settlements, risk factors of IPIs such as low socioeconomic level, none or primary school attendance level, difficulties of access to safe water and inadequate sanitary conditions, are common and favor the intestinal parasite transmission. The prevalence of IPIs among apparently healthy adults was 34.8%. This prevalence of pathogenic IPIs is high when we considered that most of the reports from Gabon include nonpathogenic species such as E. coli, E. nanus, or other non-intestinal parasites [21, 26]. Thus, these results confirm that adults constitute an important parasite reservoir of IPIs in Gabon. The frequency of intestinal polyparasitism was 36.4%, as high as the one found in Gabonese children in 2018 (41.5%) [22]. Adegnika et al reported higher rate of polyparasitism several years ago in pregnant women (65%) [27]. In Nigeria, this prevalence of intestinal polyparasitism was lower, 27.6% [1]. Living in rural areas was associated with higher rate of polyparasitism, as well as higher rates of STHs-protozoa coinfection carriage. Poor knowledge on STH transmission patterns was linked to low school attendance, agriculture practice conditions, lack of access to safe drinking water and the use of non-conventional latrines, which are frequent in rural settings and also constitute risk factors for STH, thus it would explain the observed association of polyparasitism and rural sites [3,10,28-30]. Other risk factors are contact with wet soil during agriculture and fishing activities, which are common in villages. The association of rural living conditions and polyparasitism was reported elsewhere [31,32]. According to parasites species, the same trends was noticed. Urban dwellers had less frequent polyparasitism, which involved STHs. STHs multiple species carriage was more frequent among rural inhabitants. Although different type of IPIs coinfections according to the communities are reported by other, the living conditions of rural inhabitants of our study population maintain his exposure to the different parasite species [33-35]. There is an urgent need to confirm these observations among adults from other part of the country, in order to design appropriate control strategies, which will target this age group, which is not included in deworming programs. Moreover, the impact of the intestinal multiple parasite chronic carriage on anemia, on nutritional status, on intestinal microbiome diversity and on inflammation status of adults should also be investigated.

This study has some limitations: there was only one stool sample collected per participant, and the molecular methods, which are more sensitive than the microscopy were not used. Thus, the number of parasites species as well as the prevalence of polyparasitism can be under estimated. Moreover, the cross-sectional design of the study cannot allow to determine the length of the parasitism and the fact that IPI participants were not recently

infected. Nevertheless, four different microscopic techniques were used for the parasite detection, and this study was performed using tools available in most of the IPI endemic settings that lack of molecular technology. These results can be compared with those generated locally in other settings. Furthermore, investigations on apparently healthy non-pregnant adults are also lacking in Gabon and in other IPIs endemic areas. Therefore, this study provides baseline information useful for the policy makers and the national control programs.

Conclusion

Intestinal polyparasitism is frequent in adults living in urban and rural settlements of Gabon. Adults should be also targeted by the actual intervention strategies, as they would maintain the pathogenic intestinal parasites transmission. Larger longitudinal studies with multiple sample collection per participant, using molecular methods and which investigate the impact of polyparasitism in adult's health should be performed.

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