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Blastocystis sp. Infection in Gabon: Prevalence and Association with Sociodemographic Factors, Digestive Symptoms and Anaemia

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Abstract: Introduction: *Blastocystis* sp. is an intestinal protozoan that is commonly reported, but whose clinical significance remains controversial. Clinical forms of this infection range from asymptomatic carriage to clinical signs, specifically gastrointestinal ones. There is a lack of data on the epidemiology of this protist in Gabon. This study was carried out to provide data on the frequency of *Blastocystis* sp. infection and its association with clinical signs and the haemoglobin rate. **Methods:** A cross-sectional study was conducted between September 2018 and November 2019. Stool samples were collected in five of the nine provinces of Gabon from children and adults. Sociodemographic and clinical data were recorded using a standardised pre-tested questionnaire. Haematological parameters and temperature were reported in a laboratory register. Parasitological diagnosis was performed using stool direct examination and a Merthiolate-Iodine-Formaldehyde (M.I.F) concentration to detect *Blastocystis* sp. **Results:** In total, 843 participants were interviewed and examined; 414 brought back stool samples. The frequency of *Blastocystis* sp. infection was 45.2% (n = 187/414), and it increased with age: from 20.0% in young children to 49.5% in adults (P = 0.0057). Being a male (P = 0.08) tended to be associated with *Blastocystis* sp. carriage. In the multivariate logistic regression, only males were associated with *Blastocystis* sp. infection and had a 4.3-fold higher risk of being infected than females did (adjusted odds ratio = 4.3; 95% CI = 1.2–15.6; P = 0.03). Diarrhoea, abdominal pain and colitis were observed in some patients with *Blastocystis* sp. monoinfection. No relation between *Blastocystis* sp. carriage and anaemia was found. **Conclusion:** The frequency of *Blastocystis* sp. infection was high. Males were more at risk of being infected. *Blastocystis* sp. could be used as indicator in the improvement of environmental sanitation and hygiene, coupled with improved housing. Additional investigations in a population with clinical symptoms should be performed.

Keywords: *Blastocystis* sp.; monoinfection; Gabon; risk factors; clinical signs; anaemia

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1. Introduction

Blastocystis sp. is a unicellular, eukaryotic, anaerobic protist that is detected in the gut of many hosts, such as birds and mammals. It is the most common intestinal parasite found in human faeces, whatever their age [1]. This stramenopile has a wide polymorphism according to size and form: it has vacuolar (the most recognised form using a light microscope), amoeboid, granular and cysts forms.

This parasite has been reported worldwide. In 2006, *Blastocystis* sp. was introduced in the list of water-borne parasites by the World Health Organization and was found in one billion of people throughout the world [2].

Blastocystis sp. is considered to be a commensal organism due to the asymptomatic carriage reported among populations [3]. However, evidence attribute to this protist clinical symptoms, such as pruritus, colitis, diarrhoea, irritable bowel syndrome, and also, colorectal cancer [4–6].

In developing countries, the burden of *Blastocystis* sp. infection may reach 60.0% or more [7]. In these countries, the risk factors related to the faecal–oral transmission of *Blastocystis* sp. have been identified, such as age, poor sanitation, being in close contact with animals, the consumption of contaminated drink and/or food, the use of latrines, living with infected persons, having a low household income and parents' education [7–11]. The tropical climate is also correlated with a high prevalence of this parasite [12]. In Malaysian villages, the protist has been found in water [13].

In Gabon, a retrospective study performed between 2004 and 2014 showed an increase in the prevalence of *Blastocystis* sp., ranging from 0.7 to 45.6% among symptomatic individuals. Indeed, some previous studies reported the high prevalence of *Blastocystis* sp.-infected individuals, 41.6% and 48.6%, among populations living in rural areas and in shanty towns of Libreville, respectively [1,14–16]. Many neighbourhoods lack water supplies in Libreville, the capital city of Gabon, exposing populations to faecal–oral transmission and to *Blastocystis* sp. infection. However, the relationship between the presence of this parasite and clinical symptoms was not investigated in the country.

Furthermore, a lower rate of haemoglobin among individuals infected with *Blastocystis* sp. has been reported [17–19]. Anaemia is an important public health problem in Gabon [20]. It affects 62.5% and 58.9% of young children and non-pregnant women aged 15-49 years old, respectively (https://www.indexmundi.com/facts/gabon/prevalence-of-anemia). The impact of malaria and intestinal helminthiasis, such as *Ascaris lumbricoides*, *Trichuris trichiura* and *Necator americanus*, on anaemia occurrence have been described, but no researchers have investigated that of *Blastocystis* sp. [21,22]. Thus, the aim of the present study was to assess the relationship between *Blastocystis* sp. infection, sociodemographic factors, clinical signs and the haemoglobin rate.

2. Methods

2.1. Study Sites, Period and Populations

A cross-sectional study was conducted from September 2018 to June 2019 in five out of the nine provinces of Gabon in sites where a project on the epidemiology of malaria/helminthiasis/intestinal

protozoan coinfection was conducted [1,23]. Populations living in areas with different levels of urbanization, urban, suburban and rural areas, were recruited. Socioeconomic, demographic and clinical data were collected during face-to-face interviews with populations and reported using a standardised case report form. Each participant brought a single stool sample, as recommended by the study staff. Infections with intestinal parasites, malaria and filariasis were also recorded. Axillary temperature (°C) and haemoglobin rate (g/dL) when available, and parasitological data were reported using the case report form. The haemoglobin rate was measured using the Hemocue Hb 201+ Analyzer (Ängelholm, Sweden). Thick and thin blood smears and nested PCR were performed for the detection of *Plasmodium* sp. infection [24,25]. Direct blood examination and the leukoconcentration technique, direct stool examination, Merthiolate-Iodine-Formaldehyde coloration and concentration tests were performed for filariasis and intestinal parasites infections diagnosis, respectively, as previously described [1,14].

2.2. Procedures of Data Selection

The database was created on Microsoft Excel® using data obtained from the case report forms of each participant. Double data entry was performed by two independent operators. After data cleaning, the database was duplicated: one version was conserved, and the second one was re-opened and modified for the present study.

Data from participants found with malaria, blood filariasis and intestinal parasites other than blastocystosis and co-infections were not considered to avoid bias (Figure 1).

2.3. Ethics

This study received ethical clearance from “Comité National d’Ethique pour la Recherche” (CNER) of Gabon PROT No 003/2016/SG/CNE. The protocol and the questionnaire were also approved by the Ministry of Health. Infected patients were treated according to the national guidelines.

2.4. Statistical Analysis

Statistical analyses were performed using Statview 5.0 (SAS Institute, Inc., Cary, NC, USA) and R v4.3.2 software. Qualitative variables such as “gender”, “age groups”, “locations”, “marital status”, “illiteracy”, “education level”, “type of house”, “occupation”, “type of toilet”, “open water body near home”, “regular wearing of shoes when outside”, “source of drinking water”, “fever”, “digestive symptoms”, “anaemia”, “type of anaemia” and “*Blastocystis* sp. monoinfection” are presented in percentages (%). Quantitative variables were examined for normal distribution and kurtosis and skewness in applied parametric or non-parametric tests. The quantitative variables, “temperature” and “haemoglobin level”, followed a normal distribution, and are presented as means ($\pm$ standard deviation). Participants were categorised by age into less than 5 years old, 5-15 years and more than 15 years old under the “age groups” variable. The sex ratio (male/female) was calculated. Populations were distributed among four groups according to the haemoglobin rate: absence of anaemia, mild anaemia, moderate anaemia and severe anaemia (https://www.who.int/vmnis/indicators/haemoglobin.pdf). The “temperature” variable was transformed into a binary variable; those with a fever were placed in the “yes” group if the value was above of 37.5 °C or the “No” group if it was below this.

The comparison of *Blastocystis* sp. infections according to the characteristics of the population was performed with Pearson’s Chi2 test, and haemoglobin rates and temperatures were compared with Student’s t-test.

Crude odds ratios (cORs) and 95% confidence intervals (CIs) were used to assess the association between “*Blastocystis sp*” monoinfection and “gender”, “age groups”, “locations”, “marital status”, “illiteracy”, “education level”, “type of house”, “occupation”, “type of toilet”, “open water body near home”, “wearing of shoes”, “source of drinking water”, “digestive symptoms”, “anaemia” and

"type of anaemia". Logistic regression was performed to estimate the adjusted odds ratios (aOR) between "gender", "age groups", "locations" and "marital status" and those with a *P* value <0.20 in the bivariate analysis. A *P* value <0.05 was considered to be statistically significant.

3. Results

3.1. Participants' Characteristics

During the study, 843 participants were enrolled and interviewed; among them, 414 brought back stools samples (Figure 1).

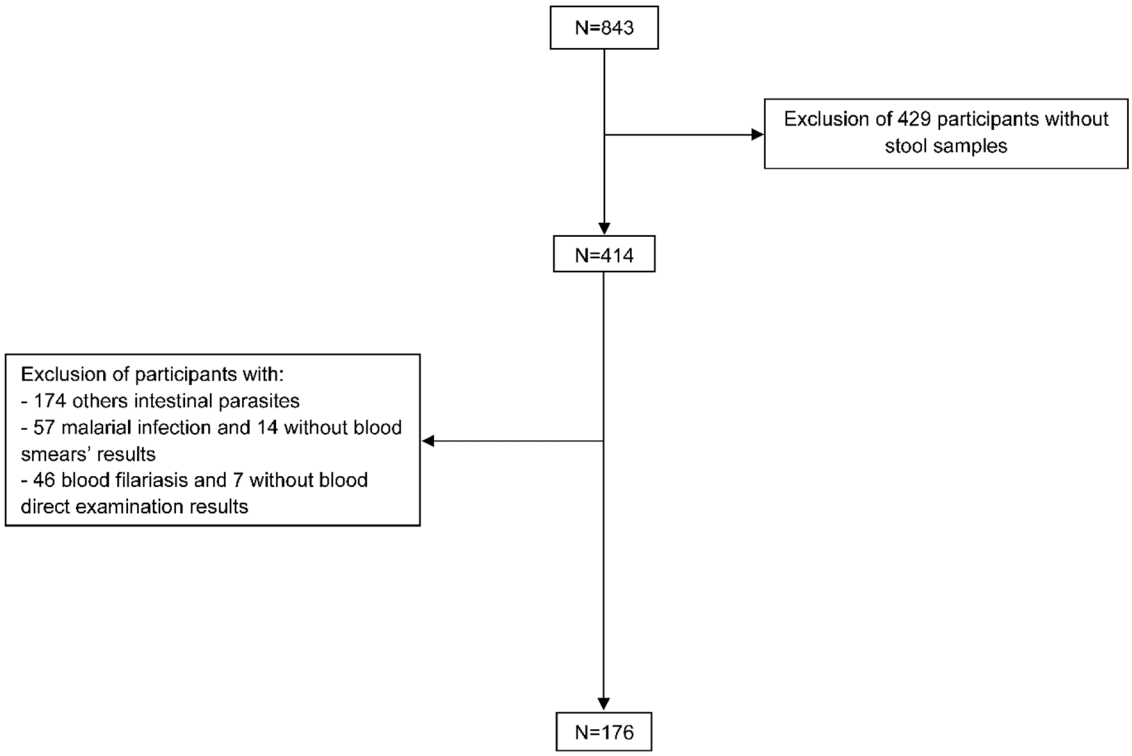

Figure 1: Flow diagram of the study population selection. This figure shows the selection process of the study population. First, participants with no stool samples were excluded. After this, we were left with all participants infected with other parasites: intestinal parasites infection, malaria and blood filariasis. Additionally, individuals who did not provide results of the biological test were excluded.

The characteristics of participants (n = 414) whose brought back stool samples were examined, as summarised in Table 1. The rate response to the questionnaire items ranged from 61.4% (n = 254/414) to 97.3% (n = 403/414). Nearly two-thirds of the participants were older than 15 years (62.6%; n = 234/374). Female participants were predominant; the sex ratio was 0.7. Three quarters of the participants lived in rural areas (75.1%; n = 268/357) and 40.1% were single (n = 130/324). During the interview, 17.5% (n = 26/320) declared themselves to be illiterate, 7.4% (n = 23/312) had no education qualification, and almost half of the study population was unemployed (44.4%; n = 119/298). Regarding lifestyle, more than 2/3 (68.6%; n = 177/258) lived in a wood house or in a sheet metal house or in an earthen house. In addition, more than one third of the participants used non-conventional latrines (40.5%; n = 120/296), lived near of an open water body (40.1%; n = 107/267) and did not wear shoes regularly outside (39.5%; n = 106/268). Likewise, when both

"spring" and "river" water consumption were considered, almost one-third of the participants had drunk unsafe water (30.7%; n = 78/254).

Among the febrile patients or those with a history of having a fever (56.4%; n = 84/149), the mean temperature was 37.8 ± 1.2 °C. The mean rate of haemoglobin was 10.6 ± 2.2 g/dL, and anaemia was reported in 64.6% (n = 93/144) of the participants. Moderate anaemia was predominant (44.4%; n = 64/144).

3.2. *Blastocystis* sp. Infection Frequency

The frequency of *Blastocystis* sp. infection was 45.2% (n = 187/414). Table 1 shows that among the patients with or without *Blastocystis* sp. monoinfection only (n = 176), men (P = 0.08) tended to be more frequently infected. *Blastocystis* sp. monoinfection was significantly associated with the age: it increased from 20.0% among children aged less than 5 years old to 49.5% among individuals that were more than 15 years old (P = 0.0057).

3.3. Association between Temperature, Haemoglobin Level and *Blastocystis* sp. Monoinfection

The mean temperatures were comparable among patients with *Blastocystis* sp. monoinfection (37.8 ± 1.1 °C) and uninfected ones (38.0 ± 1.1 °C) (P = 0.4). Infected patients had a higher level of haemoglobin (10.8 ± 2.0 g/dL) compared to that of the uninfected patients (9.8 ± 1.9 g/dL) (P = 0.08).

Table 1: Characteristics of the study population and distribution of *Blastocystis* sp. infection

	Overall Population n = 414		**Selected Participants n = 176**		***B.* sp. Infected Individuals n = 68**		
	n *	**%**	**n ****	**%**	**n *****	**%**	***P***
Demographic characteristics							
Age groups	**374**		**161**		**67**		
<5 years old	64	17.1	40	24.8	8	20.0	
5–15 years old	76	20.3	24	15.0	11	45.8	
>15 years old	234	62.6	97	60.2	48	49.5	0.0057
Socioeconomic characteristics							
Gender	**403**		**172**		**68**		
Male	165	40.9	78	45.3	37	47.4	
Female	238	59.1	94	54.7	31	33.0	0.08
Locations	**357**		**153**		**58**		
Urban area	34	9.5	24	15.7	10	41.7	
Suburban area	55	15.4	33	21.6	9	27.3	
Rural area	268	75.1	96	62.7	39	40.6	0.36
Marital status	**324**		**142**		**57**		
Single	130	40.1	53	37.3	26	49.1	
Family ****	194	59.9	89	62.7	31	34.8	0.13
Literacy	**320**		**141**		**56**		
No	56	17.5	20	14.2	7	35.0	
Yes	264	82.5	121	85.8	49	40.5	0.8
Education level	**312**		**139**		**56**		
No education	23	7.4	11	7.9	2	18.2	
Primary school	123	39.4	46	33.1	20	43.5	
Middle school	148	47.4	71	51.1	31	43.7	
High school	18	5.8	11	7.9	3	27.3	0.3

Table 1: *Cont.*

	Overall Population n = 414		**Selected Participants n = 176**		***B.* sp. Infected Individuals n = 68**		
	n *	**%**	**n ****	**%**	**n *****	**%**	***P***
Type of house	**258**		**101**		**41**		
Brick house	59	22.9	33	32.7	13	40.6	
Wood house	155	60.1	51	50.5	19	37.2	
Mixed house	22	8.5	9	8.9	6	60.0	
Sheet metal house	7	2.7	3	3.0	0	0.0	
Earthen house	15	5.8	5	4.9	3	60.0	0.2
Occupation	**298**		**116**		**44**		
Middle manager	43	16.1	22	19.0	6	27.3	
Senior manager	7	2.6	4	3.4	2	50.0	
Employee	86	32.1	42	36.2	21	50.0	
Unemployed	119	44.4	45	38.8	15	33.3	
Retired	13	4.8	3	2.6	0	0.0	0.2
Type of toilet	**296**		**110**		**44**		
Modern	133	45.0	45	40.9	13	28.9	
Non-conventional latrine	120	40.5	63	57.3	30	47.6	
Conventional latrine	43	14.5	2	1.8	1	50.0	0.1
Open water body near home	**267**		**112**		**44**		
Yes	107	40.1	53	47.3	23	43.4	
No	160	59.9	59	52.7	21	35.6	0.5
Regular wearing of shoes when outside	**268**		**113**		**45**		
Yes	162	60.5	69	61.1	27	39.1	
No	106	39.5	44	38.9	18	40.9	1.0
Source of drinking water	**254**		**111**		**44**		
Spring	21	8.3	8	7.2	3	37.5	
River	48	18.9	11	9.9	5	45.4	
Tap	176	69.3	88	79.3	34	38.6	
River+tap	7	2.7	2	1.8	2	100.0	
Spring+tap	2	0.8	2	1.8	0	0.0	0.4
Clinicobiological characteristics							
Fever	**149**		**69**		**21**		
Yes	84	56.4	43	62.3	10	23.2	
No	65	43.6	26	37.7	11	42.3	0.16
Digestive symptoms	**77**		**26**		**7**		
Yes	37	48.1	17	65.4	5	29.4	
No	40	51.9	9	34.6	2	22.2	1.0
Anaemia	**144**		**38**		**10**		
Yes	93	64.6	29	76.3	8	27.6	
No	51	35.4	9	23.7	2	22.2	1.0
Type of anaemia	**144**		**38**		**10**		
No	51	37.0	9	23.7	2	22.2	
Mild	64	44.4	25	65.8	7	28.0	
Moderate	25	17.4	3	7.9	1	33.3	
Severe	4	2.8	1	2.6	0	0.0	0.9

* indicates the number of participants who brought back stool samples; ** number of participants after the exclusion of patients with malarial infection, blood filariasis and other intestinal parasites than *Blastocystis* sp.; *** *Blastocystis* sp.-infected participants; **** family: both parents lived with the children included in the study.

3.4. Digestive Symptoms and Frequency of *Blastocystis* sp.

The presence or absence of digestive symptoms was reported for 77 participants who underwent the stool examination, but in 3 cases, the symptoms were not specified. Less than half had digestive symptoms (45.9%; n = 34/74). Colitis was frequent (32.4%; n = 24/74), followed by abdominal pain (28.4%; n = 21/74) and diarrhoea (17.6%; n = 13/74) (Table 2). Out of 34 participants who had digestive symptoms, diarrhoea (20.6%; n = 7/34) was the predominant clinical sign, and abdominal pains were less frequently reported (8.8%; n = 3/34) among those with a single symptom. Among the

patients with more than one clinical symptom, the association between colitis and abdominal pains was most frequently observed (38.2%; n = 13/34) (Table 2).

No relationship was established between the presence of digestive symptoms and *Blastocystis* sp. infection ($P > 0.05$). Out of the 26 with known digestive symptoms, 7 carried this parasite. One patient had diarrhoea only, colitis associated with abdominal pains was reported in three patients, and one other participant presented with colitis, abdominal pains and diarrhoea. *Blastocystis* sp. was not found in patients with rectal pain (Table 2).

Table 2: Digestive symptoms reported by the study population and relationship with blastocystosis.

	Overall Population n = 414		**Selected Participants n = 176**		***B.* sp.-Infected Individuals n = 68**		***P***
Digestive symptoms	**N = 74 ***	**%**	**N = 26**	**%**	**N = 7**	**%**	
Colitis							
Yes	24	32.4	13	50.0	4	30.8	
No	50	68.6	13	50.0	3	23.1	1.0
Abdominal pains							
Yes	21	28.4	9	34.6	4	44.4	
No	53	72.6	17	65.4	3	17.6	0.3
Diarrhoea							
Yes	13	17.6	7	26.9	2	28.6	
No	61	83.4	19	73.1	5	26.3	1.0
Rectal pains							
Yes	1	1.3	1	3.8	0	0.0	
No	73	98.7	25	96.1	7	28.0	1.0
Association of digestive symptoms	**N = 34**	**%**	**N = 17**	**%**	**N = 5**	**%**	***P***
1 symptom							
Abdominal pains only	3	8.8	0	0.0	0	0.0	
Colitis only	4	11.8	3	17.6	0	0.0	
Diarrhoea only	7	20.6	4	23.5	1	25.0	
2 symptoms							
Colitis+diarrhoea	2	5.9	1	5.9	0	0.0	
Colitis+abdominal pains	13	38.2	6	35.3	3	50.0	
3 symptoms							
Colitis+abdominal pains+diarrhea	4	11.8	2	11.8	1	50.0	
Colitis+abdominal pains+rectal pains	1	2.9	1	5.9	0	0.0	0.5 **

* indication of the digestive symptom was missing for 3 patients; ** *P* value for the association of digestive symptoms.

3.5. Risk factors of *Blastocystis* sp. monoinfection

Bivariate analysis identified two at-risk factors associated with *Blastocystis* sp. monoinfection: being more than 15 years old (cOR = 3.9; 95% CI = 1.6–9.4; $P = 0.002$) and being 5–15 years old (cOR = 3.4; 95% CI = 1.1–10.3; $P = 0.03$). Being a male (cOR = 1.8; 95% CI = 0.9–3.4; $P = 0.05$), being single (cOR = 1.8; 95% CI = 0.9–3.6; $P = 0.09$), being an employee *versus* a middle manager (cOR = 2.7; 95% CI = 0.9–8.1; $P = 0.08$) and having a fever (cOR = 2.4; 95% CI = 0.8–6.9; $P = 0.099$) tended to be at-risk factors (Table 3).

In the multivariate analysis performing using logistic regression, being a male was a risk factor of *Blastocystis* sp. infection (aOR = 4.3; 95% CI = 1.2–15.6; $P = 0.03$) (Table 3).

Table 3: Risk factors among populations with only *Blastocystis* sp. infection.

	***Blastocystis* sp. infection**			
Risk factors	**cOR (95% CI)**	***P***	**aOR (95% CI)**	***P***
Gender				
Male vs. female	1.8 (0.9–3.4)	0.05	4.3 (1.2–15.6)	0.03
Age groups				
>15 years old vs. < 5 years old	3.9 (1.6–9.4)	0.002	2.1 (0.3–13.6)	0.4
[5–15] years old *vs.* < 5 years old	3.4 (1.1–10.3)	0.03	2.3 (0.5–10.8)	0.3
Location				
Rural area vs. Suburban area	1.8 (0.7–4.3)	0.17	0.6 (0.08–4.7)	0.6
Urban area *vs.* Suburban area	1.9 (0.6–5.8)	0.26	2.2 (0.5–9.5)	0.3
Marital status				
Single vs. Family	1.8 (0.9–3.6)	0.09	1.1 (0.3–4.1)	0.9
Illiteracy				
Yes vs. No	1.2 (0.5–3.4)	0.6	.	.
Education level				
Middle school vs. Primary school	1.0 (0.5–2.1)	0.98	.	.
High school vs. Primary school	0.5 (0.1–2.1)	0.3	.	.
No education vs. Primary school	0.3 (0.06–1.5)	0.1	.	.
Type of house				
Brick house vs. Earthen house	0.4 (0.06–2.9)	0.4	.	.
Wood house vs. Earthen house	0.4 (0.06–2.6)	0.3	.	.
Mixed house vs. Earthen house	1.3 (0.1–12.8)	0.4	.	.
Occupation				
Senior manager vs. Middle manager	2.7 (0.3–23.4)	0.4	.	.
Employee vs. Middle manager	2.7 (0.9–8.1)	0.08	.	.
Unemployed vs. Middle manager	1.3 (0.4–4.1)	0.6	.	.
Type of toilet				
Non-conventional latrine vs. Conventional latrine	0.9 (0.05–15.2)	0.9	.	.
Modern vs. Conventional latrine	0.4 (0.02–7.0)	0.5	.	.
Open water body near home				
No vs. Yes	0.7 (0.3–1.5)	0.4	.	.
Digestive symptoms				
Yes vs. No	1.4 (0.2–9.6)	0.7	.	.
Fever				
No vs. Yes	2.4 (0.8–6.9)	0.099	.	.
Anaemia				
No vs. Yes	0.7 (0.1–4.4)	0.7	.	.
Type of anaemia				
Mild vs. Moderate	0.8 (0.06–10.0)	0.8	.	.

4. Discussion

This study is the first one to report the epidemiology of *Blastocystis* sp. monoinfection in different areas of Gabon and its relationship with sociodemographic data and clinical symptoms in Gabonese populations.

The prevalence of *Blastocytis* sp. was 45.2%, as previously described among patients consulting at the Department of Parasitology and Mycology among populations living in the 3rd and the

6th districts of Libreville, Gabon [14]. The prevalence was lower in Egypt (26.5–34.5%), Iran (7.0%), Thailand (7.2–27.4%) and Malaysia (10.6–25.7%) [9–11,18,19]. However, in Sub-Saharan Africa some authors from the Ivory Coast (58.2%), Nigeria (84.0%), Senegal (100.0%) and in Central Africa, in Cameroon (88.2%), reported a higher prevalence of *Blastocystis* sp. [26–29]. In these studies, molecular tools were used for *Blastocytis* sp. diagnosis that may explain these differences. Indeed, molecular tools are twofold more sensitive than microscopy is for the detection of stramenopile [2,12,30,31]. This observation suggests that the prevalence of *Blastocystis* sp. infection in Gabon may be underestimated.

Others factors could contribute to the variation in *Blastocystis* sp. prevalence, such as the presence of symptoms, being old and being HIV-positive according to serology examination [9,11,32]. Indeed, in Equatorial Guinea, a neighbouring country of Gabon, the parasite was more frequently detected among people living with HIV who did not received ART treatment [32]. *Blastocystis* sp. was also found in Gabon and Ghana among people living with HIV, although less frequently than it was found among HIV-negative participants [15,33].

In the current study, the prevalence of *Blastocystis* sp. infection increased with age. In surveys performed in Thailand and in Nigeria, similar results were observed [9,28]. These data suggest the role of adults as a reservoir of *Blastocystis* sp., such as it has been reported for other intestinal pathogens (soil-transmitted nematodes, *Giardia intestinalis*, *Cryptosporidium* sp., *Isospora belli* and *Entamoeba histolytica*). Indeed, in an orphanage at Chypre, infants living with parasitised adults were more at risk of infection [9]. After adjusting for confounding factors in multivariate analysis, no relationship between age and the carriage of *Blastocystis* sp. was observed.

On the other side, male had a fourfold higher risk of infection than their female counterpart did. This relationship has not been reported elsewhere [28,34,35]. Multivariate analysis was carried out on a smaller sample size in comparison to that of the population at baseline. Additionally, the following variables, “educational level”, “occupation” and “fever”, were not included in the multivariate analysis due to missing data. It is possible that occupation may be a factor favouring the frequent infection of men. Additionally, other risk factors for blastocystosis was not considered in the present study, such as living with domestic animals or water being treated before consumption [36].

The main intestinal symptom in the current study was abdominal pain, as reported elsewhere, followed by colitis, diarrhoea and rectal pain [37,38]. Others gastrointestinal disorders, such as bloating, constipation, flatulence and vomiting, have also been related to *Blastocystis* sp. infection [37,38]. However, in Senegal, in Chypre, in Australia and in the present study, no relationship was found between the presence of the parasite and the occurrence of these symptoms [26,30,39].

Blastocystis sp. has been related to anaemia [17–19,40–42]. An explanation for this may be that *Blastocystis* sp., such as other enteric parasites, can lead to a possible decrease in the absorption of nutrients in the intestinal mucosa due disturbing leptin and adiponectin levels in children [43]. However, in the present study, anaemia was not related to *Blastocystis* sp. infection. It is possible that the small sample size may explain these results. Other haematological parameters may be explored, such as erythrocyte sedimentation rate and C-reactive protein, the levels of which were found to be higher in cases of *Blastocystis* sp. infection compared to those of a control group. Inversely, levels of haematocrit, leukocytes, neutrophils were found to be lower [17,41]. This study contains interesting information that shows that *Blastocystis* sp. can cause inflammation and may have an impact on the immunomodulation of the human host. This should be explored.

5. Conclusion

Blastocystis sp. prevalence in Gabon is high and indicates the poor hygiene and living conditions of populations. The intestinal parasite has been found more frequently among elderly individuals and in the male population, but the analysis was performed on a small sample size, which is the main limitation of the present study. Health education should be implemented in the country to also control soil-transmitted helminthiasis and achieve elimination by 2030. In addition to the risk factors, the

identification of knowledge, attitudes and practices via running an adapted sensibilization campaign would have a positive impact on faecal and intestinal parasite transmission. *Blastocystis* sp., the intestinal parasite that is more prevalent in the country, must be considered as an environmental indicator to assess the efficiency of different integrated strategies that will be deployed in the world.

Author Contributions: M.K.B.-A. was the principal investigator and conceived the study with N.P.M. and D.P.M.-M. N.P.M. collected data in the field; biological sample collections and slides reading were performed by N.P.M., J.V.K.L., R.M., J.M.N.N. and B.P.N. Physical examinations and clinical data processing was performed by F.B.B.O. D.P.M.-M. and N.P.M. wrote the paper. M.K.B.-A. and O.A.M.N. reviewed and edited the paper. The statistical analyses were carried out by N.P.M. and D.P.M.-M. took part in the interpretation of data. All authors read and approved the final manuscript.

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Abbreviations
There are no non-standard abbreviations.

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