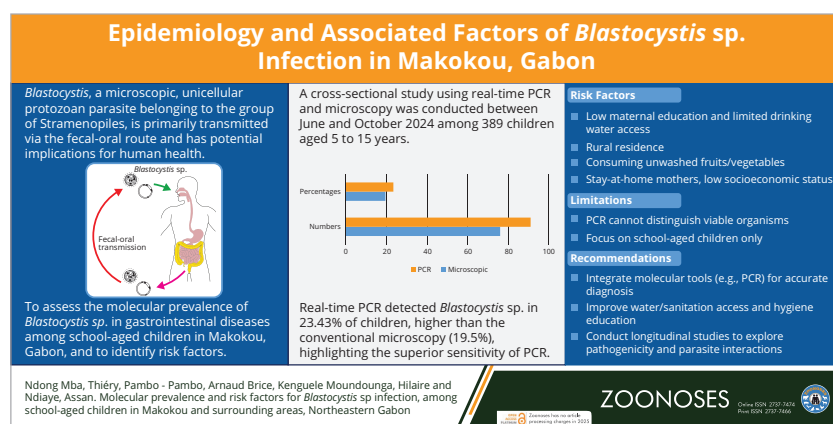




ORIGINAL ARTICLE

Molecular Prevalence and Risk Factors for *Blastocystis* sp. Infection Among School-Aged Children in Makokou and Surrounding Areas, Northeastern Gabon

Graphical abstract



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Brief statement

This study provides valuable insights into the prevalence and risk factors of *Blastocystis* sp. infection among schoolchildren in rural Gabon, highlighting its public health importance and calling for targeted prevention strategies in resource-limited tropical settings.

Prevalence comparison by diagnostic method (microscopy vs. PCR). The prevalence of *Blastocystis* sp. detected by real-time PCR (23.43%) was significantly higher than that determined through conventional microscopy, thus highlighting the superior sensitivity of molecular methods in identifying this parasite.

Highlights

The present study's strengths include the following:

- Rigorous methods, application of advanced molecular techniques such as real-time PCR, accurate identification of associated risk factors, and provision of actionable recommendations for public health improvement.
- Valuable insights that may inform targeted interventions in resource-limited tropical settings, and enhance understanding of *Blastocystis* sp. and its potential effects on children's health.

Molecular Prevalence and Risk Factors for *Blastocystis* sp. Infection Among School-Aged Children in Makokou and Surrounding Areas, Northeastern Gabon

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Abstract

Objective: This study was aimed at assessing the prevalence of infection with *Blastocystis*, an intestinal protozoan parasite, among school-aged children, and to identify associated risk factors. Although the role of *Blastocystis* in gastrointestinal symptoms remains debated, it may pose public health concerns, particularly in children with weakened immune systems.

Materials and methods: A cross-sectional study was conducted between June and October 2024 among children 5–15 years of age in Makokou and surrounding areas of northeastern Gabon. A real-time polymerase chain reaction (PCR) detection kit was used to screen for *Blastocystis* in stool samples after written informed consent was obtained from the children's parents or guardians.

Results: A total of 389 school-aged children were enrolled in this study. The overall molecular prevalence of *Blastocystis* sp. infection was 23.43% (95% confidence interval (CI): [0.19–0.28]). Multivariate analysis revealed several factors significantly associated with elevated odds of infection: having a mother with a low education level (adjusted odds ratio [aOR] = 6.39; 95% CI [3.16–12.95], $p < 0.001$), having a mother who were daily worker (aOR = 2.18; 95% CI [1.17–40.5], $p = 0.039$), living in rural areas (aOR = 2.65; 95% CI [1.56–4.51], $p < 0.001$), belonging to a household with a low standard of living (aOR = 1.81; 95% CI [1.5–30.99], $p = 0.024$), having limited access to safe drinking water (aOR = 6.39; 95% CI [3.16–12.95], $p < 0.001$), and occasionally consuming poorly washed fruits and vegetables (aOR = 2.82; 95% CI [0.99–8.2], $p = 0.046$).

Conclusion: *Blastocystis* sp. infection is a major public health issue among children in Gabon, many of whom lack awareness regarding the infection and its transmission routes. Health education initiatives targeting hygiene practices and prevention strategies, particularly those focused on decreasing exposure to water contaminated by domestic animals, are urgently needed.

Key words: prevalence, risk factors, school-aged children, *Blastocystis* sp., Makokou, Gabon

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Received: March 15 2025

Revised: April 29 2025

Accepted: June 3 2025

Published Online: July 11 2025

INTRODUCTION

Blastocystis, a microscopic, unicellular protozoan parasite belonging to the group of Stramenopiles, has attracted increasing scientific interest because of its potential implications in human health [1]. *Blastocystis*, like many intestinal parasites, is transmitted primarily via the fecal-oral route, usually through ingestion of contaminated food or water, or zoonotic contact with animals [2]. Once inside the host, it colonizes the human gastrointestinal tract, where it may interact with the gut microbiota and influence various physiological processes [3]. The presence of *Blastocystis* is frequently associated with gastrointestinal symptoms such as diarrhea, abdominal pain, and bloating [4]. However, its role as a true pathogen remains debated, because it is often detected in asymptomatic individuals; therefore, a clear causal link between its presence and clinical disease manifestations is difficult to establish [5]. A molecular study conducted on *Blastocystis* subtypes in hospitalized patients with gastrointestinal symptoms in northern Spain has identified 44 currently recognized subtypes according to genetic variations in the small subunit ribosomal RNA (SSU rRNA) gene [6]. However, according to a more recent 2024 study, the officially accepted number of subtypes remains approximately 28, although inclusion of additional subtypes (ST18–ST28) has been proposed and remains under scientific review [7]. This extensive genetic diversity may partially explain the parasite's variable pathogenicity and the differing clinical outcomes associated with infection. The epidemiology and pathogenesis of *Blastocystis* sp. show marked differences between developed countries, where the prevalence is 0.5%–23.1%, and developing countries, where the prevalence can reach 100%. These differences are highly influenced by demographic, socioeconomic, and environmental factors [8]. For instance, poor hygiene practices, limited access to clean drinking water, and improper food handling have been identified as key contributors to the transmission of this parasite. Despite widespread infection occurrence globally, data regarding the distribution and risk factors associated with *Blastocystis* infection, particularly among children, remain scarce in many African regions, including Gabon [9]. School-aged children are particularly vulnerable to parasitic infections, because of their immature immune systems and frequent engagement in behaviors such as playing in potentially contaminated environments. Understanding the local epidemiology of *Blastocystis* is essential for designing and implementing effective public health strategies. This understanding is necessary in school settings, where parasitic infections can markedly affect children's overall health, growth, and educational performance [10]. This study was aimed at determining the molecular prevalence of *Blastocystis* sp. and identifying associated risk factors among school-aged children in Makokou and surrounding areas in northeastern Gabon. Our focus on this specific region contributes to filling a critical knowledge gap regarding the burden of *Blastocystis*

infection in Central Africa and provides valuable insights for guiding targeted public health interventions aimed at improving children's digestive health. Furthermore, our results may serve as a foundation for future research on the transmission dynamics of *Blastocystis*, its interactions with other intestinal pathogens, and the development of more effective control strategies in the region.

MATERIALS AND METHODS

Study area and participants

The study was conducted in Makokou, the regional capital of Ogooué-Ivindo province in northeastern Gabon (Fig 1). Situated within the equatorial rainforest zone, Makokou has an estimated population of approximately 16,600 inhabitants. The town is accessible via the N4 national highway and by boat along the Ivindo River. The local economy relies primarily on logging, subsistence agriculture, and small-scale trade. The region is known for its rich biodiversity, including the protected areas of Ivindo National Park, and is home to several indigenous communities, particularly Pygmy populations. Despite its ecological and touristic potential, the area faces substantial public health challenges, partly because of the presence of disease-transmitting insect vectors. These environmental and socioeconomic conditions make Makokou and its surrounding areas an important setting for studying parasitic infections such as *Blastocystis* sp.

Study type and population

This descriptive and analytical cross-sectional study was conducted to estimate the point prevalence of *Blastocystis* infection and identify associated risk factors among school-aged children (5–15 years) of both sexes attending primary and secondary schools in Makokou and the surrounding areas. The study population included students from the Ogooué-Ivindo province and parts of the Haut-Ogooué region in northeastern Gabon.

Inclusion and exclusion criteria

Children 5–15 years of age, who were enrolled in schools within Makokou or its surrounding areas, and who provided stool samples after written parental consent, were eligible for inclusion in the study. Participants were excluded if they had received antiparasitic treatment in the month before the study; had chronic conditions, such as cancer, intestinal disorders, or immunodeficiency; had acute diarrhea at the time of enrollment; had parents or guardians who declined participation; or submitted inadequate or unsuitable stool samples.

Study design and sampling procedure

The study was performed in Makokou, Ogooué-Ivindo Province, Gabon, between June and October 2024. School-aged children were recruited on a voluntary basis after community sensitization efforts by local liaison officers. Participation required written informed consent

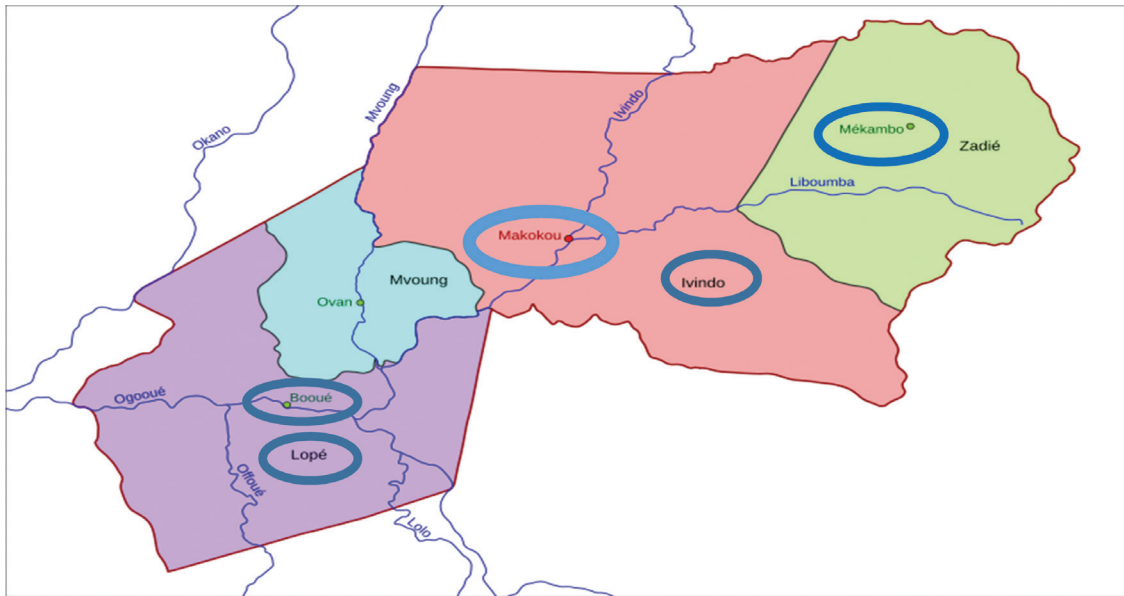


FIGURE 1 | Map of study sites (Makokou and its surrounding areas in Gabon) [11].

from a parent or legal guardian. A structured questionnaire was administered to collect sociodemographic and clinical data, as well as data on participants' knowledge and perceptions regarding *Blastocystis* sp. infections. All responses were kept confidential. Each child was given a sterile container and clear instructions for collecting a stool sample at home.

Sample size determination

To determine the appropriate sample size, we used the standard formula for estimating proportions [12]:

$$n = (Z\alpha / 2)^2 (P(1 - P)) / (d)^2$$

where

n = required sample size

$Z = 1.96$ (standard normal deviation for a 95% confidence level)

P = estimated proportion (set at 50% because of the lack of prior data for the study area)

d = margin of error (set at 5%)

On the basis of this calculation, the minimum required sample size was 292 participants. To account for potential participant dropout, non-response, or incomplete data entry, we increased the sample size by 10%, thus resulting in a target of 389 participants.

Data collection

Before the main data collection phase, a semi-structured questionnaire was pilot-tested in Booué, a region known to have high prevalence of *Blastocystis* infection. This pilot test involved 50 parents or guardians older than 18 years, with varying education levels. On the basis of feedback, the questionnaire was refined for clarity, relevance,

and ease of use. The final version covered the following domains: demographic and socioeconomic characteristics, living environment, personal hygiene practices, gastrointestinal symptoms, household sanitation (availability of toilets and running water), and lifestyle habits (e.g., hand-washing and wearing shoes). Parents or guardians completed the questionnaire; those with limited literacy were assisted individually during the process.

Stool sample collection and processing

Sample collection

Two types of biological samples, whole blood and stool, were collected from each participant. Whole blood (2 mL) was drawn and placed into EDTA tubes for complete blood count analysis, and into heparin tubes for testing of urea, creatinine, and electrolytes. Parents were also provided with a sterile container to collect stool samples from the children at home. Samples were transported to the laboratory and subjected to direct microscopic examination followed by molecular analysis with a *Blastocystis hominis* + *Dientamoeba fragilis* Real-Time PCR Detection Kit (Jant Pharmacal Corporation), according to the manufacturer's instructions.

Direct examination

Each stool sample was first examined macroscopically for consistency, color, and the presence of blood or mucus. A small portion was then processed and stained with a Kop-Color II commercial staining kit, which enhances parasite visualization against a blue background. Microscopic observation was performed with an appropriate magnification and a blue filter to increase detection accuracy.

Diagnosis by qPCR

Detection of *Blastocystis* sp. was performed with real-time polymerase chain reaction (PCR) targeting a specific

region of the SSU rRNA gene, with a previously described method [13]. The following primers were used:

Forward primer (Blast 505–532): 5'-GGA GGT AGT GAC AAT AAA TC-3'

Reverse primer (Blast 998–1017): 5'-TGC TTT CGC ACT TGT TCA TC-3'

Fluorescent probe: 5'-/56-FAM AGTTCATTAGTCA AAAATTGTTGTT/3IABkFQ/-3'

The 25 µL reactions consisted of: 5 µL DNA extracted from stool, 12.5 µL SYBR Green qPCR Master Mix (2×), 0.5 µL of each primer (10 µM), and 6.5 µL nuclease-free water. Amplification of a 479-bp fragment confirmed the presence of *Blastocystis* sp.

Statistical analysis

Data were entered into Microsoft Excel and verified before analysis in R statistical software (version 3.6.1). Continuous variables are summarized with means and standard deviations, whereas categorical variables are expressed as frequencies and percentages. Bivariate analysis was performed to assess associations between independent variables (potential risk factors) and the dependent variable (*Blastocystis* sp. infection). Variables showing statistically significant associations were further analyzed with multivariate logistic regression to identify independent risk factors, with adjustment for confounding variables. A p -value ≤ 0.05 was considered statistically significant, corresponding to a 95% confidence interval (CI).

Quality assurance

Data quality was ensured through standardized procedures, including pretesting of questionnaires on 5% of the sample and training of data collectors. An integrated quality control system was implemented at the Omar Bongo Ondimba Regional Hospital in Makokou. All laboratory analyses were performed according to standardized protocols to ensure reliability and reproducibility.

Ethical considerations

The research protocol was reviewed and approved by the Ethics Committee of the Omar Bongo Ondimba Regional Hospital Center in Makokou under reference N°0345/PHO/SG/DRS NE/SGP/D. Because the study involved minors, written informed consent was obtained from all parents or legal guardians before participation. Age-appropriate verbal or written assent was also obtained from the children themselves when feasible. The study objectives, methods, potential risks and benefits, and duration were clearly explained to both the parents and children. Participant confidentiality and anonymity were strictly maintained throughout the study. All participants were informed of their right to withdraw voluntarily at any stage without consequence.

RESULTS

Overall prevalence of *Blastocystis* sp. infection among schoolchildren included in the study (N = 389)

This study, conducted in Makokou (northeastern Gabon) between June and October 2024, included a total of 389 school-aged children, of whom 91 were diagnosed according to a positive test for *Blastocystis* sp. by real-time PCR. This finding corresponded to a molecular prevalence of 23.43% (95% CI: [0.19–0.28]).

Comparison between microscopy and real-time PCR

Microscopic detection identified 19.5% of cases as positive for *Blastocystis*. In contrast, real-time PCR confirmed a prevalence of 23.43%, thus demonstrating its superior sensitivity and reliability (Table 1).

Other intestinal parasites identified by microscopy

In addition to *Blastocystis*, the microscopic examination identified several other protozoa and intestinal worms in the same children. Only 36% of the children had detectable parasites under the microscope, underscoring the overall underdiagnosis by conventional techniques (Table 2).

Specific microscopic observation: morphology of blastocystis

The cysts of *Blastocystis* sp. were identified in their characteristic vacuolar form, although a notable morphological variation was observed among the different developmental stages.

Sociodemographic factors associated with blastocystis infection among school-aged children in the study (N = 389)

Among the sociodemographic variables tested for association with *Blastocystis* infection, both bivariate and multivariate logistic regression analyses revealed statistically significant associations with several maternal and household factors. Elevated odds of infection were observed for children whose mothers had a low education level (adjusted odds ratio [aOR] = 6.39; 95% CI [3.16–12.95], $p < 0.001$), whose mothers were daily worker (aOR = 2.18; 95% CI [1.17–40.5], $p = 0.039$), who lived in

TABLE 1 | Evaluation of microscopic and molecular techniques for identifying *Blastocystis* sp. in school-aged children.

Method	Relative sensitivity	Specificity	Detected prevalence
Microscopy (Lugol after formol-acetate concentration)	Low to moderate	Moderate	19.5%
Real-time PCR	Very high	Very high	23.43%

TABLE 2 | Parasitological profile in children, according to microscopic examination.

Parasite type	Number of cases	Percentage
<i>Blastocystis</i> sp.	76	19.5
<i>Giardia lamblia</i>	24	6.2%
<i>Entamoeba histolytica</i>	10	2.6%
<i>Ascaris lumbricoides</i>	14	3.6%
<i>Trichuris trichiura</i>	9	2.3%
Hookworms	7	1.8%
No parasite detected	249	64.0%

rural areas (aOR = 2.65; 95% CI [1.56–4.51], $p < 0.001$), or who belonged to households with a low standard of living (aOR = 1.81; 95% CI [1.5–30.99], $p = 0.024$). Detailed results of the bivariate and multivariate logistic regression analyses are provided in Table 3.

Environmental and behavioral risk factors associated with blastocystis infection among school-aged children in the study (N = 389)

Using bivariate and multivariate logistic regression models, we assessed the association between *Blastocystis* infection and selected environmental and behavioral risk factors among school-aged children. In the final adjusted model, two variables showed statistically significant associations with elevated odds of infection: limited access to safe drinking water (aOR = 6.39; 95% CI [3.16–12.95], $p < 0.001$), and occasional consumption of inadequately washed fruits and vegetables (aOR = 2.82; 95% CI [0.99–8.2], $p = 0.046$) (Table 4).

DISCUSSION

Questions continue to be raised regarding the precise role of *Blastocystis* sp., a frequently detected intestinal protozoan, in gastrointestinal disease. Although this parasite is frequently identified in both symptomatic and asymptomatic individuals, its potential effects on public health, particularly among immunocompromised populations such as children, remain debated. In this study, conducted in Makokou, northeastern Gabon, the overall molecular prevalence of *Blastocystis* infection was 23.43%, in agreement with findings in other resource-limited settings. This estimate is comparable to the pooled prevalence of 24% reported in a meta-analysis of the general population in Brazil [14], lower than the 26.17% observed in Turkey [15], and substantially higher than the 9.18% reported in Spain [16]. These variations in prevalence across regions may be attributable to differences in diagnostic methods (e.g., microscopy vs. PCR), geographic and climatic conditions, age distribution, socioeconomic status, and access to sanitation and clean water [17]. Our microscopic examination of stool samples revealed that 19.5%

of children were positive for *Blastocystis* sp. according to conventional methods, whereas a positivity rate of 23.43% was confirmed through real-time PCR. This discrepancy highlights that relatively low sensitivity in detecting low parasite loads or morphologically variable stages is a key limitation of microscopy-based diagnosis [18,19]. Indeed, traditional techniques, such as Lugol staining after formol-ethyl acetate concentration, have been found to underestimate parasitic infections, because of their inability to distinguish between viable and non-viable organisms and their dependence on technician expertise [20]. In addition to *Blastocystis*, our microscopic analysis identified several other intestinal parasites, including *Giardia lamblia* (6.2%), *Entamoeba histolytica* (2.6%), *Ascaris lumbricoides* (3.6%), *Trichuris trichiura* (2.3%), and hookworms (1.8%). However, only 36% of children had detectable parasites under microscopy, thereby indicating that a high proportion of cases were undiagnosed through use of traditional methods alone. This finding underscores the importance of integrating molecular tools such as real-time PCR into routine diagnostics, particularly in epidemiological studies, given that accurate prevalence estimates are critical for public health planning [21,22]. *Blastocystis* sp. cysts were observed in their typical vacuolar forms, although morphological variability was observed across developmental stages. This variability, combined with overlapping morphologies of other intestinal protozoa, further complicates identification by microscopy, particularly in field settings where trained personnel and adequate equipment are lacking [23]. The results also highlighted several sociodemographic and environmental risk factors independently associated with *Blastocystis* infection. Children whose mothers had a low education level had 6.39 times elevated odds of infection, whereas those whose mothers were housewives showed 2.18-fold elevated odds of infection. These results are consistent with those from previous reports from Argentina [24] and Turkey [25], which similarly linked parental educational level to infection risk in children. Poor literacy and limited awareness regarding hygiene practices are likely to contribute to poor sanitation and exposure to contaminated environments [26,27]. Rural residence was identified as another significant risk factor: children living in rural areas had 2.65 times greater infection odds than their urban counterparts. This finding corroborates prior evidence of elevated transmission risks in underserved communities [28], and aligns with WHO reports highlighting a link between poverty and poor sanitation in rural settings [29]. In such areas, inadequate infrastructure often leads to unsafe water sources, improper waste disposal, and lack of hygienic facilities, all of which facilitate parasitic transmission [30,31]. In addition, children from families with a low standard of living had 1.81 times elevated infection odds, thereby confirming the previously reported influence of socio-economic determinants on infection patterns [32,33]. Limited financial resources often translate to poor nutrition, overcrowded living conditions, and limited access to

TABLE 3 | Bivariate and multivariate logistic regression of factors associated with *Blastocystis* infection, according to sociodemographic risk factors among school-aged children in the study.

Variables	Total number of patients, N (%)	Overall prevalence of <i>Blastocystis</i> sp. infection	Bivariate analysis		Multivariate analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Sex						
Male	221 (55.53)	24.43 (54/221)	1.07 [0.64; 1.78]	0.81	-	-
Female	178 (44.47)	20.8 (37/178)	Ref	-	Ref	-
Age groups (years)						
5–10	286 (73.52)	23.78 (68/286)	1.09 [0.62; 1.95]	0.9	-	-
11–15	103 (26.48)	28.75 (23/80)	Ref	-	Ref	-
Mother's education level						
Primary	77 (19.79)	33.77 (26/77)	1.93 [1.07; 3.44]	0.023	6.39 [3.16; 12.95]	<0.001*
Secondary	251 (64.52)	21.91 (55/251)	0.8 [0.48; 1.33]	0.38	2.77 [0.54; 11.9]	-
High school or above	61 (15.69)	30.43 (10/61)	Ref	-	Ref	-
Mother's professional status						
Housewife	27 (6.94)	0.15 (4/27)	Reference	-	1	-
Daily worker	54 (13.88)	35.19 (19/54)	1.98 [1.01; 3.8]	0.037	2.18 [1.17; 40.5]	0.039*
Trade worker	222 (57.07)	18.92 (42/222)	1.98 [0.34; 0.93]	0.021	2.11 [0.50; 8.91]	0.31
Civil servant	86 (22.11)	30.23 (26/86)	1.58 [0.34; 0.93]	0.11	0.80 [0.21; 2.92]	0.74
Residence						
Makokou	277 (54.46)	14.8 (41/277)	Ref	-	Ref	-
Other departments (Booué, Ivindo, Mekambo, or Lopé)	112 (45.54)	44.64 (50/112)	4.62 [2.73; 7.89]	<0.001	2.65 [1.56; 4.51]	<0.001*
Family size						
≤ 5	188 (48.33)	26.6 (50/188)	Ref	-	Ref	-
≥ 6	201 (51.67)	20.4 (41/201)	0.71 [0.48; 1.17]	0.15	0.82 [0.47; 142]	0.4
Family standard of living						
High	128 (32.9)	17.97 (23/128)	Ref	-	Ref	-
Moderate	138 (35.48)	21.01 (29/138)	0.84 [0.49; 1.41]	0.53	-	-
Low	123 (31.52)	41.86 (39/123)	1.91 [1.13; 3.2]	0.01	1.81 [1.5; 30.99]	0.024*

Abbreviations: OR = odds ratio; CI = confidence interval.

Note: Reference category is indicated as "Ref"; * = significant test.

healthcare, and consequently further increase vulnerability to infections. Limited access to water was associated with a 6.39 times higher risk of infection, reinforcing the previously described importance of water quality in disease prevention [34]. Similarly, irregular consumption of inadequately washed fruits and vegetables was associated with

a 2.82-fold elevated infection risk, as previously reported in other endemic areas [27,35]. In many households, open defecation and seasonal runoff during rainfall events can lead to contamination of food crops, particularly when proper food washing is not practiced before consumption [36]. Although some studies have not indicated significant

TABLE 4 | Bivariate and multivariate logistic regression analysis of environmental and behavioral factors associated with *Blastocystis* infection among school-aged children.

Variables	Total number of patients receiving treatment N (%)	Overall prevalence of <i>Blastocystis</i> sp. infection	Bivariate analysis		Multivariate analysis	
			Crude OR 95% CI	p-value	Adjusted OR 95% CI	p-value
Access to drinking water						
Limited	48 (12.34)	68.75 (33/48)	10.64 [5.23; 22.54]	<0.001	6.39 [3.16; 12.95]	<0.001*
Unrestricted	178 (44.47)	20.8 (37/178)	Ref	-	Ref	-
Proximity to domestic animals or livestock near dwellings						
Yes	103 (26.48)	17.48 (18/103)	0.62 [0.33; 1.12]	0.10	1.09 [0.57–2.07]	0.79
No	286 (73.53)	27.24 (73/286)	Ref	-	Ref	-
Adequate sanitation						
Yes	328 (84.32)	18.29 (60/328)	Ref	-	Ref	-
No	61 (15.68)	50.82 (31/61)	4.59 [2.49; 8.52]	0.38	-	-
Consumption of washed raw fruit and vegetables						
Always	162 (33.89)	14.81 (24/162)	Ref	-	Ref	-
Often	207 (43.31)	27.54 (57/207)	1.65 [1.0; 2.8]	0.042	-	-
Sometimes	21 (22.8)	47.62 (10/21)	3.21 [1.18; 8.66]	0.014	2.82 [0.99; 8.2]	0.046*
Knowledge of good hygiene practices						
Good	243 (50.84)	28.81 (70/243)	Ref	-	Ref	-
Average	114 (23.85)	11.40 (13/114)	0.32 [0.16; 0.63]	0.000	-	-
Poor	32 (25.31)	25 (8/32)	1.1 [0.41; 2.65]	0.82	-	-

Abbreviations: OR = odds ratio; CI = confidence interval.
 Bold format indicates statistical significance ($p < 0.05$).

associations between *Blastocystis* infection and socioeconomic or environmental variables [37], our data clearly showed that multiple contextual factors converge in shaping transmission dynamics in this region.

Limitations and methodological considerations

Despite its contributions, this study has several limitations. Its cross-sectional survey design established associations rather than causal relationships. The sample was restricted to school-aged children in Makokou and surrounding areas, thereby limiting the generalizability of the findings to broader or non-scholastic populations. Although real-time PCR is a highly sensitive diagnostic tool, it does not distinguish between viable and non-viable organisms, nor does it assess clinical symptoms directly. Longitudinal studies would provide deeper insights into the pathogenic potential of *Blastocystis* and its long-term health consequences. Moreover, the initial microscopic screening highlighted the need for complementary diagnostic

approaches in parasitology. As previously noted, microscopy alone may miss as many as 16% of *Blastocystis*-positive cases [38], and consequently might affect the interpretation of prevalence trends and delay targeted interventions. Our findings support the growing consensus that molecular tools should be integrated into surveillance systems, particularly in tropical and resource-limited settings where mixed infections are common [39].

CONCLUSION

This cross-sectional study revealed a substantial burden of *Blastocystis* sp. infection among school-aged children in northeastern Gabon, with a molecular prevalence of 23.43%. Key risk factors identified include low maternal education, rural residence, low family socioeconomic status, and limited access to safe drinking water. These findings underscore the urgent need for targeted public health interventions, particularly in rural areas, to improve sanitation, promote hygiene education, and ensure access

to clean water. Public health programs should prioritize community-based awareness campaigns focusing on preventive behaviors such as handwashing, proper food handling, and safe water consumption. Furthermore, the integration of molecular diagnostics into routine screening protocols is essential for improving case detection and decreasing underreporting, particularly in regions with high parasitic co-endemicity. Finally, longitudinal studies are warranted to better understand the clinical relevance of *Blastocystis* and evaluate the effectiveness of preventive strategies in decreasing infection rates and improving children's health outcomes.

DATA AVAILABILITY STATEMENT

Because of a recent viral attack on our data management system, some datasets used in this study have been lost. Unfortunately, complete access to the raw data are therefore not ensured. Limited or partial data, as available, may be obtained from the corresponding author on reasonable request.

ETHICS STATEMENT

The study protocol was approved by the Ethics Committee of the Omar Bongo Ondimba Regional Hospital Center of Makokou under reference N°0345/POI/SG/DRS NE/SGP/D (N°0345/Ogooué-Ivindo Province/General Secretariat/NorthEast Regional Health Directorate/Category D). Written informed consent was obtained from all parents or legal guardians before participant inclusion, and child assent was obtained when appropriate.

AUTHOR'S CONTRIBUTIONS

This study was conceptualized by HKM and led by TNM. TNM, AN, and BAPP contributed to the study design, data analysis, and drafting of the Materials and Methods section. TNM and BAPP prepared the initial draft of the manuscript, which was critically reviewed and revised by TNM, HKM, and AD. All authors read and approved the final version of the manuscript for submission.

ACKNOWLEDGEMENTS

The authors thank all participating children and their families for their cooperation. We also extend our gratitude to the Director General of the Omar Bongo Ondimba Regional Hospital Center of Makokou, as well as the staff of the medical laboratory, for their technical support. Special thanks are due to the Regional Director of Health North-East in Makokou for facilitating the study's ethical approval process.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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