Lower prevalence of Blastocystis sp. infections in HIV positive compared to HIV negative adults in Ghana

Veronica Di Cristanziano¹, Rossella D’Alfonso², Federica Berrilli³, Fred Stephen Sarfo⁴,⁵, Maristella Santoro³, Lavinia Fabeni⁶, Elena Knops¹, Eva Heger¹, Rolf Kaiser¹, Albert Dompreh⁵, Richard Odame Phillips⁴,⁵,⁷, Betty Norman⁴,⁵, Torsten Feldt⁸, Kirsten Alexandra Eberhardt⁹

¹ Institute of Virology, University of Cologne, Faculty of Medicine and University Hospital of Cologne, Cologne, Germany, ² Department of Systems Medicine, University of Rome Tor Vergata, Rome, Italy, ³ Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, Rome, Italy, ⁴ Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, ⁵ Komfo Anokye Teaching Hospital, Kumasi, Ghana, ⁶ National Institute for Infectious Diseases L. Spallanzani—IRCCS, Rome, Italy, ⁷ Kumasi Center for Collaborative Research in Tropical Medicine, Kumasi, Ghana, ⁸ Clinic of Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Düsseldorf, Germany, ⁹ Department of Tropical Medicine, Bernhard Nocht Institute for Tropical Medicine and I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

* k.eberhardt@bnitm.de

Abstract

Background

Sub-Saharan Africa is endemic for intestinal parasites and distinguished for the largest burden of HIV cases. Blastocystis sp. is one of the most common protists infecting humans but its role in human disease is still controversial. Aim of this study was to investigate the prevalence of Blastocystis sp. in HIV positive and negative adults in Ghana and its association with immune status and other risk factors.

Methods

122 HIV positive outpatients and 70 HIV negative blood donors from the Komfo Anokye Teaching Hospital in Kumasi, Ghana, were included in the present study. Demographic, clinical and laboratory data were collected and HIV positive patients distinguished for CD4+ T cell count <200 cells/μl (n = 54) and >200 cells/μl (n = 68). A Blastocystis’s phylogenetic analysis was performed to determine sample subtype (ST).

Results

The prevalence of Blastocystis sp. in adult HIV positive individuals was lower than in HIV negative persons (6.6% vs. 20.0%, p = 0.008) and Blastocystis sp. ST1 was the most prevalent strain. Within HIV positive participants, the prevalence of Blastocystis sp. was lower in those individuals with CD4+ T cell count <200 cells/μl than in patients with higher CD4+ T cell count (1.9% vs. 10.3%, p = 0.076). Multiple regression analysis revealed that Blastocystis sp. was inversely associated with an obese Body Mass Index (BMI) in HIV negative...
persons ($p = 0.040$). Presence of *Blastocystis* sp. was correlated with higher CD4+ T cell count in HIV positive participants ($p = 0.049$).

**Conclusion**

It is largely reported that people living with HIV (PLHIV) in Africa are affected from parasite infections and that co-infections may adversely impact on their immune status, accelerating progress to AIDS and worsening gastrointestinal manifestations. Differently, in this study *Blastocystis* sp. was associated with a better immune status jointly with a healthy body weight while it seems to be reduced with the progression of HIV infection. This data agree with recent suggestions that *Blastocystis* sp. can represent a component of the healthy gut microbiota.

**Introduction**

*Blastocystis* sp. represents worldwide one of the most common human intestinal protozoan parasites, showing prevalence higher than 5% in developed countries and much higher in developing countries [1]. However, the morphological diversity exhibited by this parasite and the lack of standardization in diagnostic techniques have led to confusion about its prevalence and role as human pathogen [2]. Originally, *Blastocystis* sp. was considered a commensal microorganism of the gastrointestinal tract. However, the increasing detection rate, largely attributable to the utilization of improved molecular methods, has raised the question as to whether *Blastocystis* sp., or different *Blastocystis* sp. subtypes, could be implicated in gastrointestinal disorders and therefore redefined as a potentially pathogenic symbiont (i.e. pathobiont).

Hence, the current knowledge on *Blastocystis* sp. is characterized by disagreement about its clinical relevance, pathogenic potential, and need of treatment, particularly in immunocompromised patients, such as subjects affected by HIV/AIDS [3–5]. Beside asymptomatic infections, the spectrum of symptoms associated with *Blastocystis* sp. includes nausea, abdominal cramps, flatulence and acute or chronic diarrhea [6, 7]. The manifestations in *Blastocystis* sp. carriers could be related to genetic differences on the subtype (ST) level [8, 9]. Indeed, the *Blastocystis* sp. sequences exhibit remarkable genetic diversity and at least 17 subtypes, among which the first nine found in humans with and without intestinal disorders, have been described based on the gene coding for the small-subunit ribosomal RNA (ssu rRNA) [10]. *Blastocystis* sp. ST1–ST4 account for 90% of all human carriage, with ST3 and ST1 appearing to be the most common subtypes [11, 12]. ST3 is reported to be the most frequent subtype detected in symptomatic patients, followed by ST1 and ST2 [8]. In Sub-Saharan Africa provision of safe water supplies, sanitation and hygiene are often inadequate and constitute risk factors that facilitate infections with feco-oral transmissible agents [13]. Furthermore, Sub-Saharan Africa counts for the majority of HIV and AIDS cases reported worldwide [14, 15].

As known, diarrhea is a major cause of morbidity in HIV-infected patients, occurring in 30–60% of AIDS patients in developed countries and in up to 90% in developing countries [14, 16, 17]. In Ghana, 300,000 people are estimated to be living with HIV/AIDS and only 70,000 of them are reported to be having access to antiretroviral therapy (ART) [18]. Recent studies have shown that the presence of parasitic infections could disturb the balance of anti-HIV immune responses and contribute to HIV replication which could accelerate progression to AIDS [19].

**Competing interests:** The authors have declared that no competing interests exist.
The aim of the present study was to deepen our understanding of the implications of Blastocystis sp. detection in persons with and without HIV-infection living in Ghana. For this purpose, we studied the differences in genotype of Blastocystis sp. and the association with the immune status, clinical symptoms, therapeutic treatments, and intestinal co-infections.

Material and methods

Study design and study population

Between November 2011 and November 2012, consecutive adult HIV positive patients presenting to the HIV outpatient Department of the Komfo Anokye Teaching Hospital in Kumasi, Ghana, and HIV negative blood donors from the same hospital were recruited for a prospective observational cohort study on clinical and sociodemographic determinants of H. pylori co-infection among HIV-infected and non-infected individuals [20, 21]. To analyse the prevalence, risk factors and clinical implications of Blastocystis sp. co-infection, participants were randomly selected from HIV positive patients with CD4+ T cell counts below or more than 200 cells/μl, and from HIV negative individuals from the above mentioned observational cohort. One stool sample from each participant was tested for Blastocystis sp. and other common enteric pathogens.

This study was carried out in accordance with the World Medical Association’s Declaration of Helsinki. The protocol was approved by the Committee on Human Research of the Kwame Nkrumah University of Science and Technology in Kumasi, Ghana: CHRPE/AP/12/11, and the ethics committee of the Medical Council in Hamburg, Germany: PV3771. Written informed consent was obtained from all participants before enrolment.

Data collection and laboratory methods

Demographic and clinical data were collected by trained study personnel using a standardized questionnaire. Blood samples were collected and the analysis of CD4+ T cell count was performed in Ghana using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, California). Aliquots of native stool samples were freshly frozen and stored at -80°C before being transported to Germany on dry ice.

700 μl of a 10% suspension in Phosphate-Buffered-Saline (PBS) of each stool sample was prepared and nucleic acids were extracted from this suspension by using the automated platform VERSANT kPCR Molecular System and the VERSANT Sample Preparation 1.0 Reagents Kit (Siemens Healthcare Diagnostics), according to the manufacturer’s instructions.

Blastocystis sp. detection and subtyping

In order to detect the presence of Blastocystis sp., the small subunit rRNA (SSU-rDNA) 600 bp fragment amplification was carried out by using primers RD5—BhRDr while a second amplification was performed using the primers Blasto 2F and Blasto 2R according to the protocol as previously described [22, 23]. The PCR amplicons, after visualization by gel electrophoresis, were purified and directly sequenced on both strands by the Bio-Fab Research (Rome, Italy).

A phylogenetic analysis on Blastocystis sp. Sanger sequences was performed to determine subtype and genetic variability between patients and subtypes. Briefly, the sequences were aligned with reference sequences of the nine Blastocystis sp. subtypes found to date in human (ST1-ST9). The alignment was edited using the BioEdit program version 7.0.5.3. Tree was generated using the Generalised Time Reversible (GTR) substitution model and 1,000 bootstrap replicates with maximum-likelihood (ML) method using RAxML program (https://github.com/stamatak/standard-RAxML) [24]. Pairwise genetic distances between referenced Blastocystis sp. subtypes and patient subtypes were generated using the Tajima-Nei model [25]. All
positions with less than 95% site coverage were eliminated. Therefore, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. The variation rate among sites was modelled with a gamma distribution (shape parameter = 0.5). Evolutionary analyses were conducted using MEGA6.

**Co-infections with other enteric pathogens**

In order to exclude intestinal disorders caused by other enteric pathogens, all *Blastocystis* sp. positive samples were screened for the presence of common pathogens. The total nucleic acids were extracted as previously described [26]. For each specimen, the molecular diagnostic xTAG GPP Luminex assay (Luminex Molecular Diagnostics, Toronto, Canada), including adenovirus types 40/41, norovirus genogroup I and II (GI/GII), group A rotavirus, *Campylobacter* spp., *Clostridium difficile* toxin A/B, *Escherichia coli* O157, enterotoxigenic *Escherichia coli* (ETEC) LT/ST, *Salmonella* spp., Shiga-like toxin producing *E. coli* (STEC) stx1/stx2, *Shigella* spp., *Vibrio cholerae*, *Yersinia enterocolitica*, *Cryptosporidium hominis* and *C. parvum*, *Entamoeba histolytica*, *Giardia duodenalis*, and the multiplex FTD Viral gastroenteritis (Fast-Track Diagnostics, Luxembourg), including noro-, adeno-, rota-, astro-, and sapovirus, were utilized.

**Statistical analysis**

Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) and compared using the unpaired Student’s t-test or the Wilcoxon rank sum test. Proportions were compared using either the $\chi^2$ test or the Fisher exact test, as appropriate. Multiple logistic regression models were used for assessing independent associations between demographic, medical and immunological parameters and *Blastocystis* sp. status. Two-sided p-values were presented and statistical significance was determined at $\alpha = 5\%$. Statistical analyses were conducted using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

**Results**

**Blastocystis sp. prevalence**

122 participants with HIV infection (n = 54 with CD4+ counts < 200 and n = 68 with CD4+ counts > 200 cells/μl) and 70 HIV negative individuals were enrolled from the Komfo Anokye Teaching Hospital in Kumasi. The prevalence of *Blastocystis* sp. in non-HIV infected adult participants seen was 20.0% (n = 14/70), while the rate of co-infections with *Blastocystis* sp. in HIV positive persons was significantly lower (6.6%, n = 8/122, p = 0.008, Fig 1). A tendency, although not statistically significant, mark for a lower prevalence of *Blastocystis* sp. in HIV positive subjects with CD4+ T cell count of less than 200 cells/μl than in patients with higher CD4+ T cell count (1.9% vs. 10.3%, p = 0.076).

**Phylogenetic analysis**

Out of the 22 *Blastocystis* sp. positive patients, 21 corresponding sequences were successfully obtained. For one HIV negative patient, no valid sequence was obtained. Phylogenetic analysis revealed that ST1 was the most prevalent strain (Fig 2). In particular, 14 patients were infected with ST1 strain (66.7%), 4 patients with ST2 (19.0%), and 3 infected with ST3 (14.3%).

**Characteristics of *Blastocystis* sp. positive and negative individuals**

No differences related to sociodemographic factors were observed between *Blastocystis* sp. positive and negative participants within the two subgroups of HIV positive and negative persons...
Blastocystis infection in HIV positive and HIV negative adults in Ghana

![Graph showing Blastocystis prevalence in %]

- **HIV negative**: 20.00%
- **HIV positive**: 6.56%
- **HIV positive with CD4+ > 200 cells/μl**: 10.29%
- **HIV positive with CD4+ < 200 cells/μl**: 1.85%

* indicates a statistically significant difference.
However, Blastocystis sp. positive persons without HIV infection showed lower CD4+ T cell counts [869 (IQR 783–984) vs 1051 (IQR 915–1360), p = 0.025] and more frequently a normal body weight as compared to Blastocystis sp. negative persons (Body Mass Index (BMI) between 18.5 and 25 kg/m², p = 0.016). Moreover, HIV positive patients infected with Blastocystis sp. had higher median CD4+ T cell counts than those without Blastocystis sp. co-infection [527 (IQR 367–651) vs 264 (IQR 110–489), p = 0.035].

Fig 1. Prevalence of Blastocystis sp. Prevalence of Blastocystis sp. (%) in HIV negative compared to HIV positive persons, and HIV positive persons with CD4+ T cell count higher than 200 cells/μl, compared to HIV positive persons with CD4+ T cell count less than 200 cells/μl.

https://doi.org/10.1371/journal.pone.0221968.g001

(Tables 1). Moreover, Blastocystis sp. positive persons without HIV infection showed lower CD4+ T cell counts [869 (IQR 783–984) vs 1051 (IQR 915–1360), p = 0.025] and more frequently a normal body weight as compared to Blastocystis sp. negative persons (Body Mass Index (BMI) between 18.5 and 25 kg/m², p = 0.016). Moreover, HIV positive patients infected with Blastocystis sp. had higher median CD4+ T cell counts than those without Blastocystis sp. co-infection [527 (IQR 367–651) vs 264 (IQR 110–489), p = 0.035].

Fig 2. Blastocystis sp. subtype distribution.

https://doi.org/10.1371/journal.pone.0221968.g002
13 (59%) of all 22 Blastocystis sp. positive participants were co-infected by other gastrointestinal pathogens. Gastrointestinal symptoms were only reported by four participants co-infected with other pathogens (Table 2) and of these, only one was HIV positive. Almost all of Blastocystis sp. positive individuals with other gastrointestinal co-infections (n = 11, 91.67%) were infected by Blastocystis sp. ST1. In contrast, only one third of Blastocystis sp. positive participants without other pathogens were carrying ST1 (33.33%, p = 0.009). Detected co-infections were by adenovirus, ETEC, STEC, Shigella spp., Salmonella spp., and norovirus GII (single infection n = 12, multiple infections n = 1).

In our previous work, we found the prevalence of H. pylori infection to be inversely correlated with the degree of immunosuppression, a relation that was similarly seen for Blastocystis sp. infections in the present study. However, the prevalence of H. pylori was not different in
Blastocystis sp. positive and negative participants within the HIV positive and negative subgroups (62.5% vs 54.9%, p = 0.731 in HIV-positives, and 92.9% vs. 88.2%, p = 0.621 in HIV-negatives) and comparable to our previous findings in the full cohort (51.5 and 88.0%) [20].

HIV-related characteristics of Blastocystis sp. positive and negative individuals

Although CD4+ T cell counts differed significantly between HIV positive persons with and without Blastocystis sp. co-infection, no statistically significant differences in time since diagnosis of HIV-infection, intake of antiretroviral treatment, time since initiation of antiretroviral therapy, co-trimoxazole prophylaxis or rifampicin intake during the last 6 months were detected between them (Table 3). All but one patients on ART were receiving first-line therapy, either Zidovudine or Tenofovir with Lamivudine and either Efavirenz or Nevirapine and there was no correlation between drug combinations and Blastocystis sp. status observed in our data.

Factors associated with Blastocystis sp. infection

In the group of HIV positive Blastocystis sp. positive participants the CD4+ T cell count was the only risk factor positively associated with Blastocystis sp. co-infection in the simple logistic
regression model (Table 4). Also when adjusting (aOR) for other potential risk factors, such as gender, age, BMI, and current treatment in our multiple logistic regression model, *Blastocystis* sp. co-infection remained significantly associated with higher CD4+ T cell counts (aOR 1.22, 95% CI 1.00–1.50, *p* = 0.049).

The simple logistic regression model revealed that a BMI > 25 was inversely associated with *Blastocystis* sp. infection in the HIV negative group. Also after adjusting for gender, age and CD4+ T cell count in a multiple logistic regression model, higher BMI values were inversely correlated with *Blastocystis* sp. harboring (aOR 0.17, 95% CI 0.02–0.78, *p* = 0.040).

Table 3. Comparison of parameters related to HIV-infection between *Blastocystis* sp. positive and negative HIV positive participants.

| Parameters                              | HIV positive (n = 122) | Blastocystis sp. positive (n = 8) | Blastocystis sp. negative (n = 114) |
|-----------------------------------------|------------------------|-----------------------------------|------------------------------------|
| Time since diagnosis of HIV infection in months, mean ±SD | 36.5 ± 39.77 | 24.26 ± 31.41 |
| ART intake, n (%)                       | 3 (37.50)              | 51 (44.74)                       |
| Time since initiation of ART in months, mean ±SD | 63.67 ± 28.22 | 40.11 ± 26.47 |
| Co-trimoxazole intake, n (%)            | 4 (50.00%)             | 35 (30.70)                       |
| Rifampicin intake, n (%)                | 2 (28.57)              | 10 (9.90)                        |
| Intake of other antibiotics, n (%)      | 0 (0)                  | 1 (0.88)                         |

ART = Antiretroviral Therapy

https://doi.org/10.1371/journal.pone.0221968.t003

Table 4. Factors associated with the presence of *Blastocystis* sp. in HIV positive and negative persons.

| Variable                                     | HIV positive, n = 122 | HIV negative, n = 70 |
|----------------------------------------------|-----------------------|----------------------|
|                                              | Simple regression model | Multiple regression model | Simple regression model | Multiple regression model |
| Age in years/10                              | 0.69 (0.27–1.60)       | 0.87 (0.36–1.94) | 1.18 (0.75–1.80) | 1.24 (0.70–2.30) |
| Gender                                       | 1                     | 1                    | 1                     | 1                    |
| Male                                         | 0.37 (0.02–2.17)       | 0.53 (0.02–4.30) | 0.94 (0.26–3.14) | 0.68 (0.14–2.99) |
| BMI (kg/m^2)                                 | < 25                  |                      | >25                  |                      |
|                                              | 2.20 (0.43–9.63)       | 1.36 (0.20–7.66) | 0.18 (0.02–0.76) * | 0.17 (0.02–0.78) * |
| CD4+ T cell count in count/μl /100           | 1.20 (1.00–1.43) *     | 1.22 (1.00–1.50) * | 0.82 (0.65–0.99) | 0.86 (0.65–1.09) |
| Receiving ART                                | NA                    |                      | NA                   |                      |
| No                                           | 1                     | 1                    | NA                   | NA                   |
| Yes                                          | 0.56 (0.09–3.19)       | NA                   | NA                   |                      |
| Intake of Rifampicin                         | NA                    |                      | NA                   |                      |
| No                                           | 1                     | 1                    | NA                   | NA                   |
| Yes                                          | 2.57 (0.13–18.29)      | 2.95 (0.13–30.24)    | NA                   | NA                   |
| Intake of Co-trimoxazole                     | NA                    |                      | NA                   |                      |
| No                                           | 1                     | 1                    | NA                   | NA                   |
| Yes                                          | 2.26 (0.51–10.05)      | 2.22 (0.43–11.60)    | NA                   | NA                   |

*p* < 0.05

BMI = Body Mass Index; ART = Antiretroviral Therapy; NA = Not available

https://doi.org/10.1371/journal.pone.0221968.t004
Discussion

The prevalence of intestinal parasite infections reaches up to 95% in HIV positive persons in developing countries. These infections are caused both by protozoa and helminths and their main clinical manifestation is diarrhoea [14]. Blastocystis sp. is one of the most common intestinal protozoa infecting humans [3]. Data on the prevalence of Blastocystis sp. in Africa are scarcely documented and influenced by the method of detection. Our recent survey evidenced an overall prevalence of 58% in people living in a southern department of Côte d’Ivoire [23]. Similar high prevalence based on molecular analysis was reported from Liberia (70%) and from Senegal (100%) [10, 27].

The lack of available data on the potential association of the high circulation of Blastocystis sp. and the high prevalence of HIV infections in these geographical areas spurred us to consider whether Blastocystis sp. could have an impact on HIV positive patients that are more vulnerable to parasitic infections.

If on the one hand, several investigations have been performed to define the impact of parasitic enteropathogens on HIV patients in endemic areas already, on the other hand most of them focused on the main “known” pathogenic enteric parasites, while the role of Blastocystis sp. was marginally considered [28–32]. Although these studies give varying and, sometimes contradictory, results in respect to Blastocystis sp. infections in HIV/AIDS patients concerning its prevalence and related occurrence of clinical manifestations, this parasite is still considered an opportunistic cause of diarrhoea also in these individuals [3, 4].

In the present study, an unexpected result was the overall prevalence of Blastocystis sp., which was lower as compared to our previous data in a different country in West Africa and by similar molecular approach [23]. The more contained presence of Blastocystis sp. in the present cohort could be explained by the fact that more than half of the study participants were having access to tap water, 90% were having electricity in their household and around 70% stated owning a fridge [20, 21]. Furthermore, patients enrolled in this study, as HIV+ or blood donors were normally stimulated in the outpatient clinic for safeguard good health by good hygienic practices and were regularly controlled for health status. The age of subjects could also contribute to explain this result, given that our present cohort did not include children, who are more exposed to risk of infections in areas with poor hygiene conditions [33, 34].

Considering that adenovirus was the most frequent co-pathogen detected in ST1 positive patients, that adenovirus infections are mostly been thought to be species-specific, and that ST1 is also found frequently in humans, it might be hypothesized that inter-human transmissions play a major role in our cohort. The simultaneous use of two different commercial multiplex molecular assays allowed optimizing pathogen testing. The 6 adenovirus positive samples were detected by FTD assay only. Indeed, FTD Viral Gastroenteritis enables the detection of all 57 accepted human adenovirus types, whereas Luminex assay target adenovirus 40 and 41 only, which are the most frequent types associated with acute gastroenteritis. The discordant result about the norovirus GII positive specimen seem to confirm the lower reliability of Luminex assay for norovirus detection compared to other platforms, which could depend on higher complexity of xTAG GPP [35]. On the other hand, Luminex assay can detect a broad spectrum of clinically relevant enteric pathogens, including viruses, bacteria, and parasites.

Remarkably, HIV negative patients showed a rate of Blastocystis sp. infection significantly higher than HIV positive patients (20.0% vs 6.6%, p = 0.008) in agreement with what was found in China [36]. Similar results were observed in a study by Assefa et al. for protozoa other than Blastocystis sp. in HIV positive and negative subjects from Ethiopia [30].

Even more interesting, within HIV positive subjects, the prevalence of Blastocystis sp. was higher in those individuals with CD4+ T cell counts of more than 200 cells/μl than in patients...
with lower CD4+ T cell counts (10.3% vs. 1.9%, p = 0.076). Intake of medication, time since diagnosis of HIV infection or time since initiation of ART did not differ between Blastocystis sp. positive and negative persons co-infected by HIV. This data can be considered in agreement with results obtained in a study from Cameroon by Nsagha and colleagues [37].

Additionally, Blastocystis sp. was inversely associated with overweight in HIV negative persons, also after adjusting for confounders (p = 0.016 and p = 0.040). The only Blastocystis sp. positive patient with lean weight in the HIV negative subgroup was co-infected with Salmonella spp., which likely caused the reported gastrointestinal complaints of this individual. Also in a recent study on 316 individuals from Denmark and Spain, a tendency of Blastocystis sp. to be more common in lean individuals was observed [38]. Similarly, a higher Blastocystis sp. prevalence in normal weight individuals compared with overweight and obese ones was evident in the recent review by Beghini et al. [39]. However, it is well recognized that ART represents a strong risk factor for obese body weight in PLHIV and thus, it might be an explanation for not observing an association between a healthy body weight and Blastocystis sp. carriage in our HIV positive subgroup, but instead a clear correlation between BMI and CD4+ T cell count (p = 0.005) [40]. On the other hand, we found a significant association between a healthier immune status and Blastocystis sp. in these patients. The linkage between ART, obesity, CD4+ T cells and elevated levels of inflammatory markers which are also adversely correlated with progression of HIV disease is known [41]. Taking these findings together is reasonable to hypothesize that Blastocystis sp. could be related to a good health status without causing clinically harmful effects, as evidenced by the observed association with healthy body weight in HIV negative participants and a good immune status in HIV positive patients.

However, the observed association of Blastocystis sp. with lower CD4+ T cell counts and BMI values in HIV negative adults may implicate an immunomodulatory and potential adverse effect of this protist [42]. Thus, the increase of CD4+ T cells in Blastocystis sp. positive persons with HIV infection could result from a cell-mediated immune response towards the parasitic infection.

From the identification of the ST, our results appear in agreement with all those was already found in the African continent characterized by a higher prevalence of ST1-ST3 and an uncommon presence of ST4, the latter not detected in our cohort.

The analysis of clinical manifestations revealed some interesting aspects on the pathogenicity and clinical significance of this intestinal protist. A low number of Blastocystis sp. positive individuals, including only one HIV positive person, were symptomatic but all were detected co-infected by other pathogens. Recently, Beghini et al. suggested that Blastocystis sp. is a component of the healthy gut microbiome [39]. A similar association between Blastocystis sp. and eubiosis derived from a previous investigation which highlighted a different combination of microorganisms in gut microbiota of subjects infected by different intestinal protozoa [43]. Andersen et al. proposed a similar hypothesis using a metagenomics approach [44] and Audebert et al. found a higher bacterial alpha diversity in the faecal microbiota with the presence of Blastocystis sp. [45].

The present study provides some first data on the prevalence of Blastocystis sp. in Ghana and contributes to the debate on the impact of this parasite in human disease. An association of the presence of Blastocystis sp. with a better immune status jointly with a healthier body weight as shown in our study highlights the potential role of Blastocystis sp. as component of the healthy gut microbiota. This study has some limitations. Although the study design enables to detect potential associations of Blastocystis sp. with the immune status, clinical symptoms, therapeutic treatments, and intestinal co-infections, we cannot conclude on causalities. Longitudinal studies would be required to observe alterations of immune parameters according to Blastocystis sp. status, particularly after new infection with this protist. Moreover, the more
intense application of advanced molecular approaches, including multiplexed assays and next generation sequencing, especially to stool samples coming from endemic areas, will provide further explanation regarding the clinical significance of *Blastocystis* sp. in the future.

**Supporting information**

S1 File. Prevalence of *Blastocystis* infections in Ghana dataset: Data_Di_Cristanziano_et_al. (XLSX)

**Acknowledgments**

The authors are grateful to the nurses, physicians and patients of the HIV clinic of the Komfo Anokye Teaching Hospital in Kumasi, Ghana and to ESTHER Germany for their continuous support of the hospital partnership. We thank Shadrack Osei Assibey for data entry.

**Author Contributions**

**Conceptualization:** Veronica Di Cristanziano, Rossella D’Alfonso, Fred Stephen Sarfo, Torsten Feldt, Kirsten Alexandra Eberhardt.

**Formal analysis:** Kirsten Alexandra Eberhardt.

**Investigation:** Veronica Di Cristanziano, Rossella D’Alfonso, Federica Berrilli, Lavinia Fabeni, Elena Knops, Eva Heger, Albert Dompreh.

**Methodology:** Maristella Santoro.

**Project administration:** Albert Dompreh.

**Resources:** Rossella D’Alfonso, Federica Berrilli, Rolf Kaiser, Richard Odame Phillips, Betty Norman.

**Supervision:** Rolf Kaiser, Richard Odame Phillips, Betty Norman, Torsten Feldt.

**Writing – original draft:** Veronica Di Cristanziano, Rossella D’Alfonso, Federica Berrilli, Fred Stephen Sarfo, Torsten Feldt, Kirsten Alexandra Eberhardt.

**Writing – review & editing:** Maristella Santoro, Lavinia Fabeni, Elena Knops, Eva Heger, Rolf Kaiser, Albert Dompreh, Richard Odame Phillips, Betty Norman.

**References**

1. Wawrzyniak I, Poirier P, Viscogliosi E, Dionigia M, Texier C, Delbac F, et al. Blastocystis, an unrecognized parasite: an overview of pathogenesis and diagnosis. Ther Adv Infect Dis. 2013; 1(5):167–78. https://doi.org/10.1177/2049936113504754 PMID: 25165551; PubMed Central PMCID: PMC4040727.

2. Coyle CM, Varughese J, Weiss LM, Tanowitz HB. Blastocystis: to treat or not to treat. Clin Infect Dis. 2012; 54(1):105–10. Epub 2011/11/10. https://doi.org/10.1093/cid/cir810 PMID: 22075794.

3. Roberts T, Stark D, Hankess J, Ellis J. Update on the pathogenic potential and treatment options for Blastocystis sp. Gut Pathog. 2014; 6:17. Epub 2014/05/28. https://doi.org/10.1186/1757-4749-6-17 PMID: 24883113; PubMed Central PMCID: PMC4039988.

4. Sekar U, Shanthi M. Blastocystis: Consensus of treatment and controversies. Trop Parasitol. 2013; 3(1):35–9. https://doi.org/10.4103/2229-5070.113901 PMID: 23961439; PubMed Central PMCID: PMC3745668.

5. Vitetta L, Saltzman ET, Nikov T, Ibrahim I, Hall S. Modulating the Gut Micro-Environment in the Treatment of Intestinal Parasites. J Clin Med. 2016; 5(11). Epub 2016/11/16. https://doi.org/10.3390/jcm5110102 PMID: 27854317; PubMed Central PMCID: PMC5126799.
6. Boorom KF, Smith H, Nimri L, Viscogliosi E, Spanakos G, Parkar U, et al. Oh my aching gut: irritable bowel syndrome, Blastocystis, and asymptomatic infection. Parasit Vectors. 2008; 1(1):40. Epub 2008/10/21. https://doi.org/10.1186/1756-3305-1-40 PMID: 18937874; PubMed Central PMCID: PMC2627840.

7. Tan KS. New insights on classification, identification, and clinical relevance of Blastocystis spp. Clin Microbiol Rev. 2008; 21(4):639–65. https://doi.org/10.1128/CMR.00022-08 PMID: 18854485; PubMed Central PMCID: PMC2570156.

8. Parija SC, Jeremiah S. Blastocystis: Taxonomy, biology and virulence. Trop Parasitol. 2013; 3(1):17–25. https://doi.org/10.4103/2229-5070.113894 PMID: 23961437; PubMed Central PMCID: PMC3745665.

9. Yason JA, Liang YR, Png CW, Zhang Y, Tan KSW. Interactions between a pathogenic Blastocystis subtype and gut microbiota: in vitro and in vivo studies. Microbiome. 2019; 7(1):30. Epub 2019/03/11. https://doi.org/10.1186/s40168-019-0644-3 PMID: 30853028; PubMed Central PMCID: PMC6410515.

10. Alfelli MA, Stensvold CR, Vidal-Lapiedra A, Onouha ES, Fagbenro-Beyioku AF, Clark CG. Variable geographic distribution of Blastocystis subtypes and its potential implications. Acta Trop. 2013; 126(1):11–8. Epub 2013/01/03. https://doi.org/10.1016/j.actatropica.2012.12.011 PMID: 23290980.

11. Stensvold CR, Clark CG. Molecular Identification and Subtype Analysis of Blastocystis. Curr Protoc Microbiol. 2016; 43:20A.2.1–A.2.10. Epub 2016/11/18. https://doi.org/10.1002/cpmc.17 PMID: 27858971.

12. Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IK, Hossain MB, et al. Polymerase chain reaction-based genotype classification among human Blastocystis hominis populations isolated from different countries. Parasitol Res. 2004; 92(1):22–9. Epub 2003/11/04. https://doi.org/10.1007/s00436-003-0995-2 PMID: 14598169.

13. Ngure FM, Reid BM, Humphrey JH, Mbuya MN, Parija SC. Blastocystis infection in HIV positive and HIV negative adults in Ghana. PLoS One. 2015; 10(11):e0143388. Epub 2015/11/24. https://doi.org/10.1371/journal.pone.0143388 PMID: 26599971; PubMed Central PMCID: PMC4658036.

14. Pabriorne P, Phoumindr N, Borel E, Sourinphoumy K, Phaxayaseng S, Luangkhot E, et al. Intestinal parasitic infections in HIV-infected patients, Lao People’s Democratic Republic. PLoS One. 2014; 9(3):e91452. Epub 2014/03/24. https://doi.org/10.1371/journal.pone.0091452 PMID: 24662743; PubMed Central PMCID: PMC3963853.

15. Joint United Nations Programme on HIV/AIDS. The gap report. Geneva: UNAIDS; 2014. 334, 75 p.

16. Pabriorne P, Phoumindr N, Borel E, Sourinphoumy K, Phaxayaseng S, Luangkhot E, et al. Intestinal parasitic infections in HIV-infected patients, Lao People’s Democratic Republic. PLoS One. 2014; 9(3):e91452. Epub 2014/03/24. https://doi.org/10.1371/journal.pone.0091452 PMID: 24662743; PubMed Central PMCID: PMC3963853.

17. Sanchez-Aguillon F, Lopez-Escamilla E, Velez-Perez F, Martinez-Flores WA, Rodriguez-Zulueta P, Martinez-Ocaña J, et al. Parasitic infections in a Mexican HIV/AIDS cohort. J Infect Dev Ctries. 2013; 7(10):763–6. Epub 2013/10/15. https://doi.org/10.3855/jidc.3512 PMID: 24129632.

18. Mensah KA, Okeye P, Doku PN. An evaluation of a community-based food supplementation for people living with HIV in Ghana: implications for community-based interventions in Ghana. BMC Res Notes. 2015; 8:519. Epub 2015/10/01. https://doi.org/10.1186/s13104-015-1511-3 PMID: 26427622; PubMed Central PMCID: PMC4590264.

19. Tian LG, Chen JX, Wang TP, Cheng GJ, Steinmann P, Wang FF, et al. Co-infection of HIV and intestinal parasites in rural area of China. Parasit Vectors. 2012; 5:36. Epub 2012/02/13. https://doi.org/10.1186/1756-3305-5-36 PMID: 22330320; PubMed Central PMCID: PMC3310850.

20. Sarfo FS, Eberhardt KA, Domppreh A, Kuffour EO, Soltan M, Schachtscheider M, et al. Helicobacter pylori infection is Associated with Higher CD4 T Cell Counts and Lower HIV-1 Viral Loads in ART-Naive HIV-Positive Patients in Ghana. PLoS One. 2015; 10(11):e0143388. Epub 2015/11/24. https://doi.org/10.1371/journal.pone.0143388 PMID: 26599971; PubMed Central PMCID: PMC4658036.

21. Eberhardt KA, Sarfo FS, Dompreh A, Kuffour EO, Geldmacher C, Soltan M, et al. Helicobacter pylori Coinfection Is Associated With Decreased Markers of Immune Activation in ART-Naive HIV-Positive and in HIV-Negative Individuals in Ghana. Clin Infect Dis. 2015; 61(10):1615–23. Epub 2015/07/20. https://doi.org/10.1093/cid/civ577 PMID: 26195015.

22. Souppart L, Sanciu G, Cian A, Wawrzyniak I, Delbac F, Capron M, et al. Molecular epidemiology of human Blastocystis isolates in France. Parasitol Res. 2009; 105(2):413–21. Epub 2009/03/17. https://doi.org/10.1007/s00436-009-1398-9 PMID: 19290540.

23. D’Alfonso R, Santoro M, Essi D, Monsia A, Kaboré Y, Gié C, et al. Blastocystis in Côte d’Ivoire: molecular identification and epidemiological data. Eur J Clin Microbiol Infect Dis. 2017; 36(11):2243–50. Epub 2017/07/04. https://doi.org/10.1007/s10096-017-3053-1 PMID: 28674969.
24. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006; 22(21):2688–90. Epub 2006/08/23. https://doi.org/10.1093/bioinformatics/btl446 PubMed PMID: 16928733. PMID: 16928732.

25. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 1993; 10(3):512–26. https://doi.org/10.1093/oxfordjournals.molbev.a040023 PMID: 8336541.

26. Di Cristanziano V, Timmen-Wego M, Lübke N, Kaiser R, Pfister H, Di Cave D, et al. Application of Luminex Gastrointestinal Pathogen Panel to human stool samples from Côte d’Ivoire. J Infect Dev Ctries. 2015; 9(8):884–9. Epub 2015/08/29. https://doi.org/10.3855/jidc.6460 PMID: 26322882.

27. El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzy niak I, et al. Children of Senegal River Basin 27. 28. Gassama A, Sow PS, Fall F, Camara P, Gu 29. 31. Alemu A, Shiferaw Y, Getnet G, Yalew A, Addis Z. Opportuni stic and other intestinal parasites among 30. 32. Assefa S, Erko B, Medhin G, Assefa Z, Shimelis T. Intestinal parasitic infections in relation to HIV/AIDS 31. 35. Roka M, Go 36. McHugh MP, Guerendi ain D, Hardie A, Kenicer J, MacKenzie L, Templeton KE. Detection of Norovirus 37. Nsagha DS, Njunda AL, Assob NJC, Ayima CW, Tanue EA, Kibu OD, et al. Intestinal parasitic infections 38. Kotloff KL, Nataro JP, Blackw elder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiol- 39. Beghini F, Pasolli E, Truong TD, Putignani L, Cacci 40. Lake JE. The Fat of the Matter: Obesity and Visceral Adiposity in Treated HIV Infection. Curr HIV/AIDS Rep. 2017; 14(6):211–9. https://doi.org/10.1007/s11904-017-0368-6 PMID: 29043609; PubMed Centr al PMCID: PMC5694708.
41. Crum-Cianflone NF, Roediger M, Eberly LE, Vyas K, Landrum ML, Ganesan A, et al. Obesity among HIV-infected persons: impact of weight on CD4 cell count. AIDS. 2010; 24(7):1069–72. https://doi.org/10.1097/QAD.0b013e328337fe01 PMID: 20216300; PubMed Central PMCID: PMC2878190.

42. Ajjampur SS, Tan KS. Pathogenic mechanisms in Blastocystis spp.—Interpreting results from in vitro and in vivo studies. Parasitol Int. 2016; 65(6 Pt B):772–9. Epub 2016/05/18. https://doi.org/10.1016/j.parint.2016.05.007 PMID: 27181702.

43. Iebba V, Santangelo F, Totino V, Pantanella F, Monsia A, Di Cristanziano V, et al. Gut microbiota related to Giardia duodenalis, Entamoeba spp. and Blastocystis hominis infections in humans from Côte d’Ivoire. J Infect Dev Ctries. 2016; 10(9):1035–41. Epub 2016/09/30. https://doi.org/10.3855/jidc.8179 PMID: 27694739.

44. Andersen LO, Stensvold CR. Blastocystis in Health and Disease: Are We Moving from a Clinical to a Public Health Perspective? J Clin Microbiol. 2016; 54(3):524–8. Epub 2015/12/16. https://doi.org/10.1128/JCM.02520-15 PMID: 26677249; PubMed Central PMCID: PMC4767957.

45. Audebert C, Even G, Cian A, Loywick A, Merlin S, Viscogliosi E, et al. Colonization with the enteric protozoa Blastocystis is associated with increased diversity of human gut bacterial microbiota. Sci Rep. 2016; 6:25255. Epub 2016/05/05. https://doi.org/10.1038/srep25255 PMID: 27147260; PubMed Central PMCID: PMC4857090.