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BLASTOCYSTIS HOMINIS INFECTION IN CHILDREN WITH HIV/AIDS DURING COVID-19 RELATED DISRUPTION ERA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Introduction: The coronavirus disease 2019 (COVID-19) epidemic has resulted in significant disruption to health services in multiple nations. In Indonesia, the impact occurred on changes in services for HIV/AIDS patients. As a result, there is a high rate of parasitic co-infection disease, including *Blastocystis hominis*. Prior research indicates that the occurrence of this illness varies greatly among individuals with HIV/AIDS, with rates ranging from 3.86% to 72.40%. **Aims:** The objective of this study is to ascertain the prevalence and molecular epidemiology of infection, develop a predictive model, and examine the correlation between clinical symptoms and the severity of *Blastocystis* results. **Methods:** Thirty-eight children with HIV/AIDS in Yogyakarta-Indonesia, from May until August 2021 were examined by direct examination, culture, PCR, and sequencing. In addition, a structured questionnaire was used to obtain additional data regarding baseline information and other factors influencing *Blastocystis* infection. The results obtained were subjected to phylogenetic, univariate and multivariate data analysis. **Results:** Out of the 38 samples studied, 26 (68.4%) were positive for *Blastocystis*. The results of sequencing demonstrated the finding of subtype 3 (ST3) and subtype 4 (ST4). From univariate and multivariate analysis, a longer duration of therapy is a predictor of *Blastocystis* infection (AOR 6.54, P=0.04). The relationship between clinical manifestations and intensity of *Blastocystis* findings showed a non-significant association (P>0.99). **Conclusion:** Children with HIV/AIDS had a significantly high incidence of *Blastocystis* infection, potentially attributed to the interruption of services resulting from the COVID-19 pandemic.

Keywords: *Blastocystis hominis*; HIV/AIDS; COVID-19

INTRODUCTION

HIV infection continues to be a significant problem globally, with approximately 38.4 million people living with this disease at the end of 2021 and over one million children infected in the same year (World Health Organization (WHO), 2019, 2022). In Yogyakarta-Indonesia, 50 new cases were identified among children (Yogyakarta City Health Service, 2020). Furthermore, the COVID-19 pandemic since the end of 2019 has disrupted services for HIV/AIDS patients. This impacts the country's supply chain, availability, and accessibility of antiretroviral (ARVs) (World Health Organization (WHO), 2020).

Changes in health system capacity due to the COVID-19 pandemic have also increased the prevalence of co-infectious pathogens, such as *Blastocystis hominis* infection, in HIV patients. *Blastocystis* is an intestinal parasite with a complex taxonomic history, formerly categorized as fungi, and sporozoans, until classified as Stramenopiles (Clark et al., 2013; Stensvold & Clark, 2016). It can be classified as a zoonotic or enzootic infection according to its subtype (STs) (Higuera et al., 2021). A total of 32 Blastocystis subtypes have been identified, of which 10 are zoonotic subtypes (ST1-9 and ST12) (Rauff-Adedotun et al., 2021, 2022). *Blastocystis hominis* is a polymorphic organism with distinct primary and reproductive features. The wide range of morphological variations gives rise to certain diseases that are frequently overlooked in diagnosis and

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treatment. *Blastocystis* infection can be asymptomatic or symptomatic, and the morphological variations are difficult to establish because of its similarities with other species or outcomes in feces (Boorom et al., 2008; EL-Marhoumy et al., 2015; Ocaña-Losada et al., 2018). Fecal samples most commonly detect the vacuolar form of *Blastocystis*. The cyst's morphology is hypothesized to serve as an infectious phase, while its veracity remains unverified (CDC, 2019).

The previously recognized risk factors for *B. hominis* transmission include HIV stage, animal rearing, consumption of unboiled water, CD4+ T cell count, and viral load. (Albrecht et al., 1995; Zhang et al., 2019). Previous studies demonstrate that the prevalence of this infection varies significantly among HIV/AIDS patients, ranging from 3.86% to 72.40% (Kesuma et al., 2019; Zhang et al., 2019). Specific studies on *Blastocystis* infection in children with HIV/AIDS have not been conducted. Therefore, this study aims to determine the proportion and molecular epidemiology of *Blastocystis* infection, identify the subtype, determine the predictive model, and analyze the relationship between clinical manifestations and the intensity of its outcome in children with HIV/AIDS. This study provides an important contribution in understanding the genetic epidemiology of *B. hominis* in pediatric populations with HIV/AIDS during the COVID-19 pandemic, which has been associated with a period of treatment disruption. This study emphasizes the need to increase awareness regarding the potential risk of *B. hominis* infection in children with HIV/AIDS both during and after the COVID-19 pandemic. It also emphasizes the importance of taking urgent measures to avoid the spread of the infection to the wider community.

METHODS

Study Population

A cross-sectional study was conducted on a total population of 59 children that had routine check-ups at the infection and tropical diseases division of the children's outpatient ward, in Dr. Sardjito Central Hospital Yogyakarta, between May and November 2021. The sample was excluded if they refused to provide consent or declined to provide a fecal sample for the duration of the study. A questionnaire developed by Diptyanusa et al. (2021) was utilized. This questionnaire included information about the parent or guardian (seven items), the patient's baseline data (eleven items), the patient's behavior and habits (eleven items), the patient's vital signs (eight items), the patient's present symptoms (nine items), the physical examination (eleven items), and experimental parameters (three items).Patients were also asked to collect stool specimens within six hours after defecating and put in sterile containers, which were transported directly using an ice box. The specimens were examined at the Parasitology Laboratory, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. They were divided into direct microscopic examination, culture, and molecular analysis. The Ethics Committee of Gadjah Mada University has granted approval for this research, with reference number KE/FK/0182/EC/2021. The parent or guardian of the participant in this study has been provided with information on the study's objectives, as well as details about the analysis of the fecal sample, infection, and treatment. This information has been directly communicated to the concerned parent or guardian.

Microscopic

In order to carry out the direct microscopic examination, the approaches of normal saline, iodine, and formalin-ethyl acetate concentration were utilized (CDC, 2016). During the course of the investigation, a single stool specimen was obtained and placed on duplicate slides. In addition to that, the findings of the examination were validated by two other microscopists who had received training.

The intensity of *B. hominis* was determined to be negative, less than or equal to five times the high-power field (HPF), and greater than or equal to five times the HPF during the direct examination.

Culture

The Jones medium was modified by replacing horse serum with fetal bovine serum. It was then optimized to determine the concentration of fetal bovine serum to obtain optimal growth of *B. hominis*. Fifty milligrams of fresh feces samples were cultivated in Jones medium, which was modified with 20% fetal bovine serum, and then incubated at a temperature of 37ºC. Microscopic examination was used to confirm the growth of *Blastocystis* at 24, 48, and 72 hours of incubation. Finally, trichrome staining was used to visualize the positive culture results.

DNA Extraction and Amplification

The DNA was isolated from stool samples using the FavorPrep^{TM} Stool DNA Isolation Mini Kit (Favorgen Biotech Corporation, Taiwan), following the manufacturer's instructions. It was stored at -20ºC for further molecular analysis, and PCR performed an amplification to amplify the *Blastocystis* small subunit ribosomal RNA (SSU-rRNA) gene. The forward and reverse primers were 5'-ATCTGGTTGATCCTGCCAGT-3' (*RD5*) and 5'-GAGCTTTTTAACTGCAACAACG-3' (*BhRDr*), respectively (Scicluna et al., 2006). The PCR mixture was amplified in the 20 µL reaction using 10 µl PCR master mix (MyTaq^{TM} Red Mix, Bioline, UK), 6 µl PCR water, 2 µl DNA template, and 2 µl primer. The order of thermal cycling parameters used was one cycle at 95ºC for six minutes for initial denaturation, 30 cycles at 94ºC for one minute, followed by one minute of annealing at 55ºC, and one minute of extension at 72ºC. The final cycle was extended by 10 minutes at 72ºC. In addition, the PCR products underwent electrophoresis on a 2% agarose gel for a period of 30 minutes and were analyzed using a 600-bp UV transilluminator.

DNA sequencing and subtype analysis

Three amplicons derived from positive PCR products performed bi-directional single-pass DNA sequencing. The blasted sequences were aligned with previously published databases of various zoonotic *Blastocystis* STs (AB107961, AB070993, AB023499, AY590110, AB070987, AB070997, AB070988, AB107963, KX618192, AB107965, AY244620, AY135407, AB070998, AB107966, AB070990, AB070995, AB091236, AY135412, AB091244, AY590107, AB107970, KT438703, AF408426) from GenBank (National Center for Biotechnology Information) using Mega 11 software (https://www.megasoftware.net/).

The phylogenetic analysis was conducted using the Maximum Likelihood and Kimura 2-parameter statistical approach. The genetic distances were then computed using the Kimura 2-parameter model. (Kimura, 1980). Afterwards, the tree that had the highest log probability (-5552.58) was displayed.

Statistical Analysis

The final interpretation of positive *Blastocystis hominis* infection is based on a positive culture examination. According to Sari et al. (2018), this is due to the fact that it is the gold standard and demonstrates higher sensitivity than its polymerase chain reaction (PCR) counterpart. In addition, the evaluation of the data was carried out with the assistance of the IBM SPSS Statistics 26.0 software. Typically, numerical data are presented as the mean, standard deviation, or median, followed by the minimum and maximum values. In contrast, nominal data are represented as proportions.

In order to evaluate the comparison of proportions, the chi-square method was

utilized, whereas the mean or median was analyzed with either an unpaired t-test or a Mann-Whitney test. Based on the results of the univariate analysis, the risk factors that demonstrated a significance level of P<0.20 were included into the multivariate logistic regression analysis through using the stepwise technique. The variable was deemed statistically significant when the p-value in a two-tailed test was less than 0.05.

RESULT

Infection Proportion and Molecular Epidemiology of *Blastocystis hominis* Infection in Children with HIV/AIDS

Among the 59 children that were part of the study, only 38 samples underwent a thorough examination. There were twenty-one samples that were not included in the study because two of them did not provide their consent, five of them were unwilling to provide stool samples, and fourteen of them did not meet the necessary volume despite being followed up for recollection.

As can be seen in Figure 1, the flow chart for sample recruitment reveals that 68.4% of the 38 samples tested positive for the presence of *Blastocystis hominis* infection. There were 25 samples that revealed evidence of the parasite, and one of those samples showed a combination of *B. hominis* and *Entamoeba coli*. Furthermore, there were no soil-transmitted helminths (STH) present in any of the investigated samples. Phylogenetic analysis of the three PCR findings is displayed in Figure 2, which compares the results to various numbers of *Blastocystis* sequence databases. The presence of two subtypes was discovered through an analysis of the phylogenetic and genetic distance between all three positive PCR findings. These subtypes are referred to as subtype 3 (ST3) and subtype 4 (ST4).

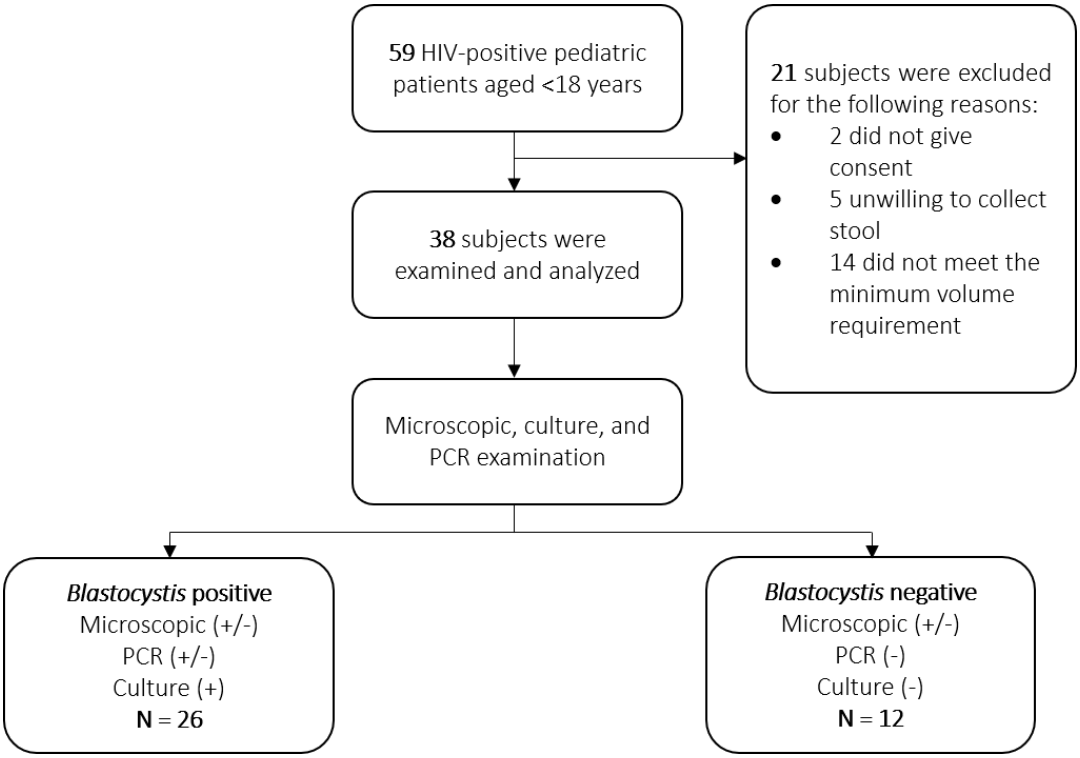

Figure 1. Study workflow diagram

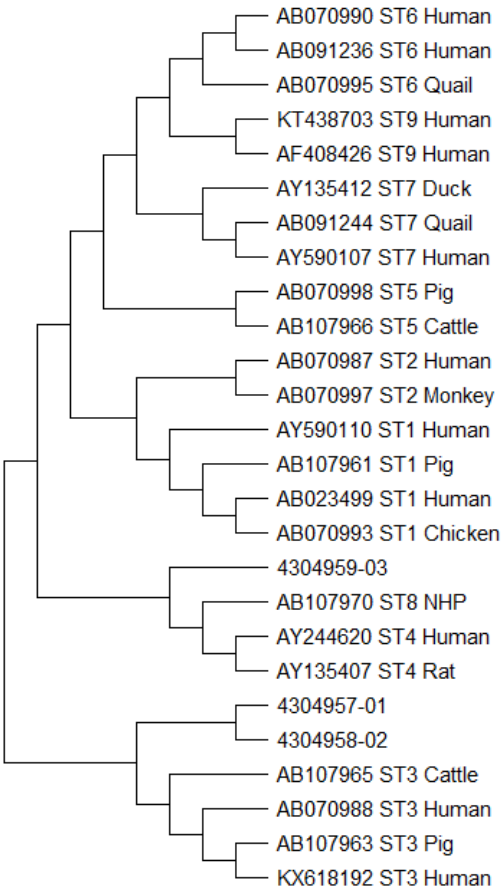

Figure 2. Phylogenetic Tree of *Blastocystis* spp. Collected from HIV/AIDS Children in Yogyakarta

Baseline Characteristic of *Blastocystis hominis* Infection in Children with HIV/AIDS

Table 1 highlights the specific characteristics of the population. This study involved 57.89% boys and 42.11% girls, dominant in the age 5-12 years. The univariate analysis revealed a statistically significant disparity in the length of therapy between the two groups (P=0.02).

Table 1. The Characteristics of the Sample and Household

Variable	Negative *n* = 12	Positive *n* = 26	*P*-value
Sample characteristics			
Gender			0.30*
Male	5 (22.7)	17 (77.3)	
Female	7 (43.8)	9 (56.3)	
Age‡	6.40 ± 3.51	6.87 ± 4.07	0.73
Age group			
Six months - 5 years	4 (30.8)	9 (69.2)	>0.99**
5 - 12 years	8 (38.1)	13 (61.9)	0.54*
13 - 18 years	0 (0)	4 (100)	0.29**
Comorbidities			
Other infection diseases	4 (26.7)	11 (73.3)	0.73**
Neurological	0 (0)	1 (100)	>0.99**

Variable	**Negative** ***n* = 12**	**Positive** ***n* = 26**	***P*-value**
None	8 (36.4)	14 (63.6)	0.70*
Duration of the diagnosis			
<1 year	3 (42.9)	4 (57.1)	0.66**
1-5 years	7 (35)	13 (65)	0.90*
>5 years	2 (18.2)	9 (81.8)	0.44**
Therapy			>0.99**
On ARV	10 (31.3)	22 (68.8)	
Not on ARV	2 (33.3)	4 (66.7)	
Duration of therapy (n=32)†	2 (0; 9)	4.6 (0.5; 10)	0.02
Duration of therapy classification (n=32)			
<1 year	2 (50)	2 (50)	0.58**
1-5 years	7 (41.2)	10 (58.8)	0.27**
>5 years	1 (9.1)	10 (90.9)	0.11**
Clinical staging			
Stage 1	4 (28.6)	10 (71.4)	>0.99**
Stage 2	2 (50)	2 (50)	0.58**
Stage 3	6 (35.3)	11 (64.7)	0.93*
Stage 4	0 (0)	3 (100)	0.54**
Immunodeficiency status			
None	5 (27.8)	13 (72.2)	0.90*
Mild	1 (20)	4 (80)	>0.99**
Advanced	3 (50)	3 (50)	0.36**
Severe	3 (33.3)	6 (66.7)	>0.99**
$CD4^+$absolute‡	667.25 ± 381.71	828.69 ± 477.37	0.31
$CD4^+$absolute status			0.32**
≤200 cell/mm^3	1 (100)	0 (0)	
>200 cell/mm^3	11 (29.7)	26 (70.3)	
Viral load ($x10^4$) (n = 29) †	0.004 (0; 8.80)	0.004 (0; 19.9)	0.75
Household Characteristics			
Job of Householder			0,91***
Government employees	1 (25)	3 (75)	
Entrepreneur/private employee	4 (23.5)	13 (76.5)	
Labor	7 (58.3)	5 (41.7)	
Farmers	0 (0)	4 (100)	
Jobless	0 (0)	1 (100)	
Education level			
Junior high school	5 (35.7)	9 (64.3)	0.73**
Senior high school	6 (35.3)	11 (64.7)	0.93*
College/University	1 (14.3)	6 (85.7)	0.40**
Income			
Low	9 (33.3)	18 (66.7)	>0.99**
Intermediate	2 (33.3)	4 (66.7)	>0.99**
High	1 (20)	4 (80)	>0.99**
Number of family members			0.27**

Variable	Negative *n* = 12	Positive *n* = 26	*P*-value
<5	2 (16.7)	10 (83.3)	
≥5	10 (38.5)	16 (61.5)	

[*] Data are analyzed using Chi-Square - Continuity Correction
[**] Data are analyzed using Chi-Square - Fisher's Exact Test
[***]Data are analyzed using Chi-Square – Linear by Linear Association
[‡] The data are shown as the mean ± standard deviation and analyzed using an unpaired T-Test.
[†] The data are displayed as a median, with the minimum and maximum values, and is then examined using the Mann-Whitney Test.

The characteristics of families and households are also shown in Table 1. Most parents/guardians are self-employed/private employees, have a high school education or equivalent, and have a low household income, accounting for 44.74%, 44.74%, and 71.05%, respectively. There were no notable disparities in the variables related to family and household characteristics between the positive and negative *Blastocystis* groups.

There were no significant differences in the symptoms and signs between the positive and negative *Blastocystis* groups. Those discovered in the *positive* group were diarrhea, nausea/vomiting, decreased drinking frequency, reduced skin turgor, and increased intestinal peristalsis. A total of two out of the 26 positive samples had extraintestinal symptoms from skin rashes. Table 2 shows clinical characteristics and physical examination.

Table 2. Clinical and Physical Examination Characteristics

Variable	Negative *n* = 12	Positive *n* = 26	*P*-value
Symptoms			
Diarrhea			0.17**
Yes	2 (15.4)	11 (84.6)	
No	10 (40)	15 (60)	
Nausea/Vomiting			0.54**
Yes	0 (0)	3 (100)	
No	12 (34.3)	23 (65.7)	
Stomach pain			N/A
Yes	0 (0)	0 (0)	
No	12 (31.6)	26 (68.4)	
Bloated			N/A
Yes	0 (0)	0 (0)	
No	12 (31.6)	26 (68.4)	
Decreased appetite			0.30**
Yes	3 (60)	2 (40)	
No	9 (27.3)	24 (72.7)	
Decreased drinking frequency			>0.99**
Yes	0 (0)	1 (100)	
No	12 (32.4)	25 (67.6)	
Decreased urination frequency			N/A
Yes	0 (0)	0 (0)	
No	12 (31.6)	26 (68.4)	
Fever			>0.99**

Variable	**Negative** ***n* = 12**	**Positive** ***n* = 26**	***P*-value**
Yes	0 (0)	1 (100)	
No	12 (32.4)	25 (67.6)	
Sign			
Sunken eyes			>0.99**
Yes	2 (33.3)	4 (66.7)	
No	10 (31.3)	22 (68.8)	
Anemic conjunctiva			0.42**
Yes	4 (44.4)	5 (55.6)	
No	8 (27.6)	21 (72.4)	
Decreased skin turgor			>0.99**
Yes	1 (25)	3 (75)	
No	11 (32.4)	23 (67.6)	
Abdominal tenderness			N/A
Yes	0 (0)	0 (0)	
No	12 (31.6)	26 (68.4)	
Increased intestinal peristalsis			>0.99**
Yes	0 (0)	1 (100)	
No	12 (32.4)	25 (67.6)	
Skin Rash			>0.99**
Yes	1 (33.3)	2 (66.7)	
No	11 (31.4)	24 (68.6)	

N/A indicates data cannot be extracted
** Data are analyzed using Chi-Square - Fisher's Exact Test

Table 3 provides a concise summary of the features of behaviors and habits. The risky behaviors and habits did not differ significantly in the two groups studied. However, a tendency toward not washing hands, and a history of close contact with animals will show a higher proportion of positive *Blastocystis*.

Table 3. Characteristics of Behavior and Habits

Variable	**Negative** ***n* = 12**	**Positive** ***n* = 26**	***P*-value**
Contact with other water sources (river/swamp/lake/pond)			0.69**
Yes	3 (37.5)	5 (62.5)	
No	9 (30)	26 (70)	
Contact with individuals who have diarrhea			0.36**
Yes	3 (50)	3 (50)	
No	9 (28.1)	23 (71.9)	
Consumption of raw vegetables and fruits			0.27**
Yes	5 (45.5)	6 (54.5)	
No	7 (25.9)	20 (74.1)	
The behavior of washing vegetables/fruits			>0.99**
Yes	12 (33.3)	24 (66.7)	

Variable	Negative *n* = 12	Positive *n* = 26	*P*-value
No	0 (0)	2 (100)	
Hand washing before eating			>0.99**
Yes	10 (33.3)	20 (66.7)	
No	2 (25)	6 (75)	
Hand washing after defecation			N/A
Yes	12 (31.6)	26 (68.4)	
No	0 (0)	0 (0)	
Close contact with animals			0.69**
Yes	9 (30)	21 (70)	
No	3 (37.5)	6 (62.5)	
History of close contact with animals			
Poultry	4 (22.2)	14 (77.8)	0.41*
Cow/Goat	2 (66.7)	1 (33.3)	0.23**
Dog/Cat	2 (40)	3 (60)	0.64**
Other	0 (0)	1 (100)	>0.99**
No contact	4 (36.4)	7 (63.6)	0.71**
Latrine presence			>0.99**
Yes	12 (32.4)	25 (67.6)	
No	0 (0)	1 (100)	
Defecating behavior			>0.99**
Toilet	12 (32.4)	25 (67.6)	
River/land	0 (0)	1 (100)	
Source of drinking water			
Tap water	0 (0)	3 (100)	0.54**
Well water	9 (33.3)	18 (66.7)	>0.99**
Bottled water	2 (28.6)	5 (71.4)	>0.99**
Rainfed water storage	1 (100)	0 (0)	0.32**
Drinking water			>0.99**
Boiled	10 (32.3)	21 (67.7)	
UV	2 (28.6)	5 (71.4)	

N/A indicates data cannot be extracted

* Data are analyzed using Chi-Square - Continuity Correction

** Data are analyzed using Chi-Square - Fisher's Exact Test

Multivariate Model for *Blastocystis* Infection in Children with HIV/AIDS

The multivariate analysis model included some variables on patient characteristics (duration of therapy and diarrhea symptoms). The findings of the multivariate model are displayed in Table 4. The predictor of *Blastocystis* infection is the duration of ARV consumption (*AOR*=6.54 (95% *CI* 1.09-39.20), *P*=0.04), with the median duration in the positive and negative groups being 4 and 2 years, respectively.

Table 4. Multivariate Model for *Blastocystis* Infection in Children with HIV/AIDS

Variable	Univariate Analysis		Multivariate Analysis	
	P-value	*COR* (95% *CI*)	*P*-value	*AOR* (95% *CI*)
Diarrhea	0.17	3.67 (0.67-20.19)	0.38	N/A

Variable	Univariate Analysis		Multivariate Analysis	
	***P*-value**	***COR* (95% *CI*)**	***P*-value**	***AOR* (95% *CI*)**
Duration of therapy (median years)	0.02	N/A	0.04	6.54 (1.09-39.20)

N/A indicates data cannot be extracted
COR: crude odds ratio
AOR: adjusted odds ratio

Association of *Blastocystis hominis* Infection and Clinical Symptoms

Table 5 presents the association between the presence of *B. hominis* and the clinical symptoms seen in patients. There was no significant difference in parasite intensity when measured by direct microscopy, as indicated by a lack of statistical significance (P=0.42, r=0.24).

Table 5. Correlation between Parasite Intensity and Clinical Manifestation of *Blastocystis hominis* Infection in Children with HIV/AIDS

Clinical Manifestation	*Blastocystis hominis* Intensity			*P-value*
	Negative ***n* = 10**	**<5/HPF** ***n* = 14**	**≥5/HPF** ***n* = 14**	
None	5 (26.32)	9 (47.36)	5 (26.32)	0.42‡
Yes	5 (26.32)	5 (26.32)	9 (47.36)	
Contingency coefficient = 0.24 (*P = 0.32*)				

‡ Data are analyzed using Chi-Square – Linear by Linear Association

DISCUSSION

Blastocystis hominis is an intestinal parasite common in several areas of Indonesia (Kesuma et al., 2019; Kurniawan et al., 2009; Sari et al., 2018; Yulfi et al., 2021). The study discovered Blastocystis infection in 68.4% of children with HIV/AIDS, indicating a serious issue. The results of the subtype analysis from sequencing and genetic distances examination showed the dominance of ST3 followed by ST4. These subtypes have been studied and is indeed common in Southeast Asian and Indonesian populations. Previous research has demonstrated that the prevailing strain in Southeast Asian and Indonesian people is ST3, with ST1, ST2, and ST4 following in order of dominance (Alfellani et al., 2013; Kurniawan et al., 2009; Sari et al., 2018). Studying the Blastocystis subtype and its genetic relationship with those found in other animals is essential to determine potential zoonotic infection. Finally, ST3 is mostly discovered in domestic animals including dogs, cats, and cattle, while ST4 can be observed in rodents and poultries (Stensvold & Clark, 2016). Evaluating the subtype findings discovered during the disruption phase has a significant impact on community health, particularly in relation to transmission modes that may indicate zoonotic diseases. Human actions can facilitate the transmission of disease-causing parasites through their interaction with the environment (Nii-Trebi, 2017; Skotarczak, 2018).

The findings of both univariate and multivariate analysis revealed that only the length of therapy exhibited a statistically significant difference between the two study groups. The median duration of ARV therapy in the positive and negative groups was 4 and 2 years, respectively. These show a paradoxical relationship, where a longer treatment period should improve immunodeficiency status compared to the shorter counterpart. However, the group with a longer treatment period had a higher proportion of *Blastocystis* infections. This was in

contrast to the study by Cristanziano et al. (2019), which showed no significant difference in the duration of ARV therapy in *Blastocystis* infection. This suggests that there are other causes associated with this co-infection. The WHO stated that there had been a disruption of services for HIV/AIDS patients in Indonesia due to the COVID-19 pandemic since the beginning of 2020. This has resulted in a shortage of drug stocks, a decline in the number of people undergoing medical interventions, and in some cases leading to the discontinuation of ARV treatment (World Health Organization (WHO), 2020). Therefore, these can impact the number of co-infectious diseases in patients despite receiving treatment for a longer period. If left untreated, this can potentially lead to the patient becoming a reservoir of *B. hominis* infection among the community through the fecal-oral route (Fasihi Karami et al., 2023; Raafat et al., 2021).

Clinical characteristics and physical examination findings did not significantly differ between positive and negative *Blastocystis* groups. Ocaña-Losada et al. (2018) showed that patients with *B. hominis* infection might present either asymptomatic or show intestinal and extraintestinal symptoms. The predominant extraintestinal manifestation linked to this infection is a cutaneous rash that diminishes with the administration of medication. The analysis between parasitic findings and clinical manifestations indicates that there was no significant relationship. These are similar to the cohort studies conducted to examine Blastocystis in patients with asymptomatic and symptomatic periods (Alfellani et al., 2013; Cristanziano et al., 2019). However, when the parasite demonstrate its pathogenicity, symptomatic individuals will exhibit higher parasite counts than their asymptomatic counterparts (El-Shazly et al., 2005; Herwaldt et al., 2001; Leder et al., 2005). According to El-Shazly et al. (2005), there is a higher intensity of *B. hominis* findings in patients with clinical manifestations. This follows the study, where symptomatic individuals had greater *B. hominis* intensity of ≥5/HPF than their asymptomatic counterparts (47.36% versus 26.32%).

CONCLUSIONS

Children in Indonesia who have HIV/AIDS continue to experience a significant prevalence of *Blastocystis hominis* infection, with subtypes 3 and 4 being the most frequently observed. The high rate of incidence in children can be caused by the disruption of HIV/AIDS services resulting from the depletion of ARVs as a result of COVID-19. These infections often do not cause symptoms; hence, parasite examinations need to be conducted.

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