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Original Research Article

Detection of *Blastocystis hominis* by Method of Cultivation in The Feces of Orphanage Children in Pekanbaru, Riau Province, Indonesia

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Abstract

Background: *Blastocystis hominis* is an intestinal protozoan that can infect humans and animals. The distribution coverage is very wide and is transmitted through the fecal-oral route. The incidence of blastocystosis due to *Blastocystis hominis* is higher in developing countries because it is associated with poor hygiene practice, inadequate sanitation, close contact with pets domesticated animals and or contaminated food. *Blastocystis hominis* infection can cause clinical manifestations, from asymptomatic to chronic diarrhea, depending on the *Blastocystis* subtype and the patient's immune system.

Objective: The aim of this study was to detect and determine the incidence of *Blastocystis hominis* infection in the feces of children at the Pekanbaru Orphanage using the culture method.

Methods: This research was a descriptive study with a cross-sectional design, that aims to detection of *Blastocystis* by the modified Jone's Medium culture method but using sheep's serum. The sample is the feces of children from nine orphanages in Pekanbaru. The data is shown in the form of figures and distribution frequency tables.

Results: A total of 95 children's stool samples were examined from 9 orphanages in Pekanbaru, it was found that 63 children (66.3%) were positive for *Blastocystis hominis* using the culture method, positive *Blastocystis hominis* was found more in female than male, and based on age group, 6-12 years is almost the same as in the age group 13-17 years. Generally, the source of drinking water in orphanages is refilled drinking water and all orphanages have cats as pets, and a few have chickens, birds, and goats as pets

Conclusion: High incidence of *Blastocystis hominis* (66.3%) can be detected in the feces of Pekanbaru orphanage children using the culture method.

Keywords: *Blastocystis; children; culture; orphanage*.
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INTRODUCTION

Blastocystis hominis is an intestinal protozoan that is commonly found in humans and animals. Its distribution coverage is very wide and this parasite is transmitted by the fecal-oral route. The prevalence of *Blastocystis hominis* is more in developing countries than in developed countries, in developing countries the prevalence of *Blastocystis hominis* is more than 60% while in developed countries it is 5% - 20%.[1] The high incidence of *Blastocystis hominis* in developing countries is closely related to poor personal hygiene and inappropriate sanitation, frequent exposure to or contact with pets domesticated animals, transmission occurs through food and beverage contaminated with *Blastocystis hominis.*[1,2]

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Blastocystosis or *Blastocystis hominis* infection can cause clinical manifestations from asymptomatic to symptoms of gastrointestinal disorders such as nausea, vomiting, bloating, abdominal pain, flatulence, acute and chronic diarrhea and sometimes constipation. This parasitic infection is sometimes associated with irritable bowel syndrome and inflammatory bowel disease.[3,4] Besides gastrointestinal symptoms, it has also been reported to cause dermatological symptoms such as itching and rashes of the skin along with gastrointestinal symptoms.[5] *Blastocystis hominis* is a protozoan that lives in the intestines, is a single-celled eukaryote, anaerobic. This parasite is a polymorphic protozoan because it has many forms, based on the literature in general there are four forms, namely vacuolar, granular, ameboid and cystic forms.[2,6] Vacuolar and ameboid forms are forms that are often found in feces and cultured products.[2] Based on the literature, it has been reported that there are 32 subtypes of *Blastocystis hominis.*[7] and the clinical manifestations in infected individuals depend on the subtype that infects and *Blastocystis* infection has been reported to occur in immunosuppressed patients such patients who received kidney transplants. It was also reported that *Blastocystis* was more commonly found in healthy people than people with inflammatory bowel disease and based on the literature it was also stated that people who contain *Blastocystis* in their intestines have a high bacterial diversity in their intestines, which means that the existence of *Blastocystis* will have a beneficial effect on the gut microbiota.[8] However, the effect of *Blastocystis* also varies depending on the infecting subtype, therefore *Blastocystis hominis* is still a controversy whether it is pathogenic or commensal because of the very wide genetic variation.[4]

Diagnostic examination for *Blastocystis hominis* can be carried out by direct microscopic examination but this microscopic examination is very subjective depending on the skills and experience of the examiner in identifying *Blastocystis hominis*. The size and morphology of *Blastocystis hominis* varies greatly during its life cycle because these protozoa have a variety of shapes that are sometimes very similar to other intestinal protozoa or similar to contaminants or fecal debris, this leads to misdiagnosis. Several studies have reported methods for diagnostic *Blastocystis*; direct microscopic, culture, serology (ELISA) and molecular methods by using PCR. However, the culture method is the recommended method for the diagnosis of *Blastocystis.*[2,9,10] Children who live in urban dense area with poor sanitation and inadequate hygiene behavior, eg., orphanages are children who are at risk for infection with *Blastocystis hominis.* Based on this, the researchers were interested in conducting research to detect *Blastocystis* in children's feces using the culture method.

MATERIALS AND METHODS

This research is a descriptive study with a cross-sectional design. The samples of this study were collected from the feces of children from nine orphanages in Pekanbaru City and examination of stool samples was carried out at the Parasitology Laboratory of the Faculty of Medicine, Riau University from August – October 2022. The stool that has been collected in a sterile tube is immediately taken to the Parasitology Laboratory and examined within 12 hours after the stool is excreted. Data containing the identity of the subject and the environment of the orphanage were obtained from the administrators of the orphanage. Stool examination was carried out directly microscopically and cultured. *Blastocystis* culture used modified Jone's medium,[11] but in this study horse serum was replaced with sheep serum. After being planted in the media, it was incubated at 37°C for 48 – 72 hours. The culture results were then read after 48 hours with iodine's stain and then examined with a light microscope with a magnification of 400x. This study passed the ethical review by the ethical review board for medicine and health research Faculty of Medicine, Universitas Riau (No: B/115/UN19.5.1.18/UEPKK/2022).

RESULTS

In this study, 95 samples of orphanage children's feces were collected from 9 orphanages in Pekanbaru City. The characteristics of the orphanage children who are the subjects of this study are as shown in table 1 below.

Table 1. Characteristics of the subjects

Characteristics	N (95)	Percentage
Sex		
- Male	58	61.1%
- Female	37	38.9%
Age groups		
- ≤ 5 years	5	5.3%
- 6 – 12 years	77	81 %
- 13 – 17 years	13	13.7%

Table 1 shows that most of the subjects were male (61.1%) with the largest age group being the 6-12-year-old group (81%). A total of 95 children's stools examined from 9 orphanages obtained *Blastocystis* culture results as shown in table 2 below.

Table 2. Distributions of positive *Blastocystis* based on orphanage

Orphanage	N	*Blastocystis* (+)	
		Frequency	%
1	19	12	63.2%
2	8	5	62.5%
3	6	5	83.3%
4	10	6	60.0%
5	4	4	100%
6	12	4	33.3%
7	9	7	77.8%
8	9	5	55.6%
9	18	15	83.3%
Total	95	63	66.3%

In table 2. it can be seen that from 95 stool samples examined, 63 children or 66.3% were positive for *Blastocystis hominis* by culture method.

Table 3. Distributions of positive *Blastocystis* based on sex and age groups

Variable		Culture of *Blastocystis*	
		Positive	Negative
Sex			
-	Male	37	21
-	Female	26	11
Age groups			
-	≤ 5	1	4
-	6 - 12	53	24
-	13 - 17	9	4

Based on table 3, it can be seen that there are many positive results in male or boys, but if it is based on the proportion of boys and girls, it is found that more girls are infected than boys. Based on age, the 6-12-year-old group detected more *Blastocystis hominis*, but based on the proportion of incidence of *Blastocystis hominis* infection in the age group 6-12 years it was not much different from the age group 13-17 years, namely 68.8% and 69.2%.

Figure 1 shows the results of the culture of *Blastocystis hominis* using Iodine's staining, from the image it appears that there are various sizes and shapes of *Blastocystis hominis* that grow in various shapes and most of them are vacuoles. Microscopic examination was carried out after 48 hours in culture and viewed with a light microscope with a magnification of 400x.

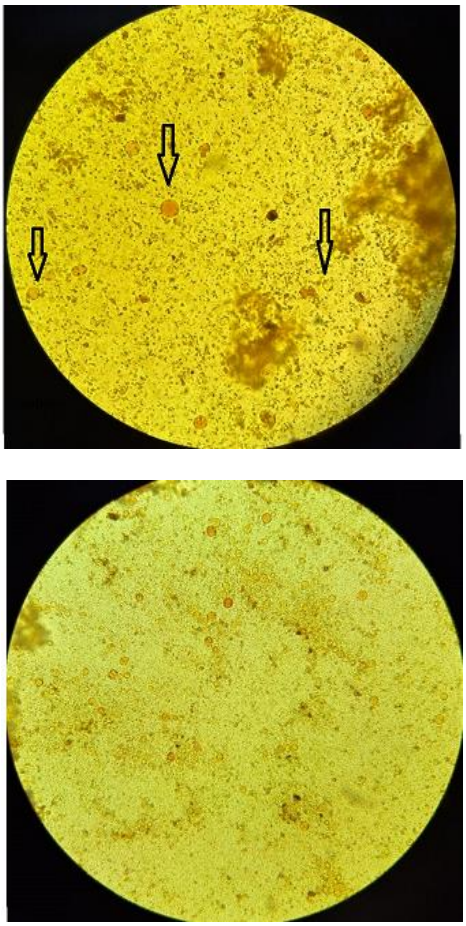

Figure 1. *Blastocystis hominis* with Iodine's stain from culture (400x magnification)

In this study, subcultures were also carried out, the results of the first culture were cultured again and after 48 hours viewed under a light microscope with a magnification of 400x. (figure 2). The picture shows that *Blastocystis hominis* has an amoeboid shape and is larger than the first culture results. Microscopic examination from culture using a light microscope with a magnification of 400x.

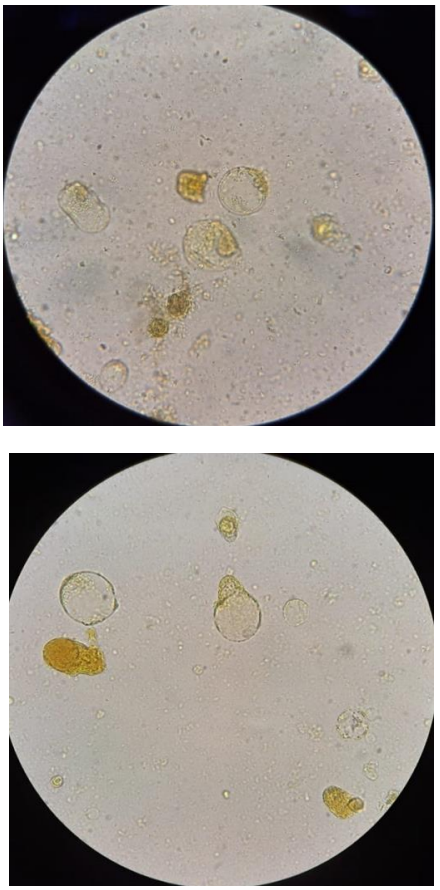

Figure 2. *Blastocystis* subculture showed amoeboid and vacuole forms using iodine's stain with a magnification of 400x

DISCUSSION

In this study, it was found that 66.3% or 63 samples from 95 children of orphanage were positive for *Blastocystis hominis* using the culture method; the high incidence of *Blastocystis hominis* infection was confirmed. In a study in Iran, 21.09% data were positive for Blastocystis in patients who did not have diarrhea.[12] In a study in Mugla, Turkey, the incidence of *Blastocystis* in school-age children was 35 out of 468 (7.4%),[13] and a 2021 study in six countries (Azerbaijan, Czechia, Jordan, Nigeria, Sudan and Tanzania) found an incidence of *Blastocystis* in children was 36%[14] and another study in Jakarta Indonesia found that the incidence of *Blastocystis* in primary school-aged children was 52.5%,[9] This is caused by many factors, possibly because orphanage children have inappropriate hygiene behavior, life in an orphanage with many children and a crowded environment and lack of attention to personal hygiene can increase the risk of being infected with *Blastocystis*. In addition, all children in the nine orphanages in this study had cats as pets. Based on the literature, cats can act as reservoir host for this protozoa, the close contact between humans and cats makes it easy for disease transmission and it can caused zoonoses.[2,15]

Blastocystis can infect male or female, in a study by Sankur et al, it was found that *Blastocystis* infection in males and females was almost the same.[13] Data from some literatures show that boys are more infected than girls.[3,16–19] The reason for this perhaps because boys prefer to play outside the house and have inappropriate personal hygiene,[17] but in this study it was found that girls were more infected. These results are also the same as studies in China and Myanmar where there were more *Blastocystis* infections in girls (7.9%) than boys (4.8%).[20] Even though based on observations most boys prefer to play with animals such as cats, chickens and goats, compared to girls who are usually afraid or feel anxious or disgusted when holding pets. Based on these results, the possibility of *Blastocystis hominis* infection in girls can be obtained from factors such as food and drink contamination by *Blastocystis* cysts and poor personal hygiene behavior.[21,22]

In this study, there was no difference in the age group infected with *Blastocystis* between the age group 6-12 years and 13-17 years, this is the same as the study in Argentina by Candela found there was no difference in intestinal parasite infection between the age group 6-12 years and the group aged 13 – 19 years.[23] Infection of *Blastocystis* is associated with several factors such as consumption of contaminated food, poor quality of drinking water, and close contact with animals, poor personal hygiene habits, and inappropriate sanitation.[3,4,14–16] The results of this study are different from studies in China and Myanmar, where the age group of 7-12 years is the age group with the most positive *Blastocystis*.[20] Based on the literature, primary school age group is the group of children who are in their peak period of playing outside the home, these primary school age children also still have less knowledge about maintaining personal hygiene so they are easily infected with parasitic infections but in this study there was no difference between age groups 6 – 12 years with 13 – 17 years.[24] All the orphanages studied had children who were positive for *Blastocystis* with different proportions ranging from 33.3% to 100%. All children examined had no symptoms or asymptomatic. *Blastocystis* is still not certain whether commensal or pathogenic and this is still controversial, based on the literature the clinical symptoms of *Blastocystis* infection are very wide ranging from asymptomatic to acute and chronic diarrhea. These symptoms depend on the infecting *Blastocystis* subtype and the human immune system.[2]

In this study, data was also collected through questionnaires about sources of drinking water and pets in the orphanage. Based on the questionnaire data provided, it was found that the source of drinking water generally came from refilled water or bottled water and only one orphanage whose drinking water came from boiled well water. Drinking water sources can be a source of *Blastocystis* infection because it is contaminated with *Blastocystis* cysts from animals or humans. From the questionnaire data on pets, it was found that all orphanages had cats as pets and most of them also kept chickens, birds and goats. Based on the literature, pets such as cats, dogs, chickens and goats are reservoir animals for this parasite and this is a source of transmission for humans.[15,25–27] In this study, *Blastocystis* examination used the culture method using modified Jones medium[11] but in this study horse serum was replaced with sheep serum because it was difficult to obtain horse serum in our region. All stages of the procedure are in accordance with what is in the literature, only the serum are different. The culture results were examined after incubation at 37°C for 48 hours and 72 hours. Examination is carried out by taking 20 ul of feces, placed on an object glass that has been given a drop of iodine, the feces are mixed evenly and covered with a deck glass and examined by microscope with 400x magnification.[11] The results are shown in figure 1. in positive stools *Blastocystis* can be seen growing in various sizes and shapes, the most of which are vacuolar and granular forms. After 3 days, the positive results were partially subcultured and read under a microscope after 2 days and the results are shown in Figure 2. The microscopic examination results showed *Blastocystis* amoeboid and vacuolar forms with larger sizes. Examination by the culture method is superior and recommended for detecting *Blastocystis* compared to direct microscopic examination, because we can immediately see the parasites growing and the number is definitely more so there is no doubt, based on the literature the sensitivity of this culture method is 90% and the specificity is 100%,[10] but the culture method takes 3-4 days compared to direct microscopic examination which can be directly examined when get a sample but need an expert because it is difficult to distinguish *Blastocystis* from yeast cells or fecal debris.[2,9]

CONCLUSION

In the feces of the children at the Pekanbaru orphanage, *Blastocystis hominis* can be detected using the culture method and this study confirmed the high incidence of blastocystosis among orphanage children. Transmission occured through fecal oral route by way of contaminated food and beverage and maintain and care for domesticated animals.

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