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Correlation of Intestinal Protozoa Infection with the Nutritional Status of Toddlers Aged 12–59 Months in Jember Regency, East Java, Indonesia

Rizky Robeth Ardyansyah,[1] Yunita Armiyanti,[2] Ida Srisurani Wijiastuti,[3] Bagus Hermansyah,[4] Wiwien Sugih Utami,[5] Erma Sulistyaningsih[6]

[1]Faculty of Medicine Universitas Jember, [2]Departement of Parasitology Universitas Jember, [3]Departement of Public Health Universitas Jember, [4]Departement of Parasitology Universitas Jember, [5]Departement of Parasitology Universitas Jember, [6]Departement of Parasitology Universitas Jember, Indonesia

Abstract

Background: Intestinal protozoan infection is a problem faced by the global community at all ages. In toddlers, it can cause problems in the form of decreased nutritional status, which is often found in developing countries such as Indonesia. The purpose of this study was to determine the correlation between intestinal protozoa infection and the nutritional status of toddlers.

Methods: An observational study with a cross-sectional approach was conducted in October–December 2022 on 45 children aged 12–59 months in Sucopangepok Village, Jelbuk District, Jember Regency, East Java, Indonesia using, consecutive sampling techniques and a total sample size. Nutritional Status was measured based on body weight to body length using the WHO Anthropometric Calculator. Stool examination used the direct smear method and modified Ziehl-Neelsen staining. Data was analyzed using the Cramer's V test. The p-value less than 0.05 was considered statistically significant.

Results: The incidence of wasted children was 15.6% and severely wasted was 2.2%. Intestinal protozoan infection had an incidence of 15.6%. The species detected were *Giardia lamblia* (6.7%), *Cryptosporidium parvum* (6.7%), and *Blastocystis hominis* (2.2%). Statistical analysis showed there was no correlation between intestinal protozoan infection and nutritional status (p= 0.441; r= 0.191).

Conclusion: There is no correlation between intestinal protozoan infection and the nutritional status of toddlers. However, comprehensive collaboration between the government and the community needs to be improved, as well as healthy lifestyles for toddlers which also need to be encouraged to overcome nutritional problems in children under five old and prevent intestinal protozoa infections.

Keywords: Intestinal protozoa, nutritional status, toddlers, wasted

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Correspondence:
Dr. dr. Yunita Armiyanti, M.Kes., Sp.ParK.,
Departement of Parasitology
Universitas Jember, Indonesia

E-mail:
yunita.fk@unej.ac.id

Introduction

Intestinal protozoan infections are a worldwide problem. It is estimated that around 280 million people each year have been infected with intestinal protozoa, which not only infect adults but also children and toddlers. A study in Mozambique[1] has shown that 11.7% of toddlers aged 12–59 months were infected with intestinal protozoa (*Cryptosporidium spp., Giardia lamblia,* and *Entamoeba histolytica)*, whereas in Brazil[2] 7.2% of toddlers were infected with *Dientamoeba fragilis*. Interestingly, studies in another part of Indonesia has shown that *Balantidium coli* (12.5%) and *Blastocystis hominis* (12.9%) frequently infects children under five.[3,4]

Intestinal protozoan infections cause problems such as decreased nutritional status in toddlers, which can be found in developing

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countries such as Indonesia.[5] The chronic nature of intestinal protozoan infection over a long period means that this problem is often overlooked, leading to impaired growth and development of children that cause a decrease in children's cognitive, achievement, and productivity.[6] Children under five who have intestinal protozoa infection and also have poor nutritional status.[7] Moreover, there is a significant relationship between *Cryptosporidium spp*. and very poor nutritional status.[1]

Nutrition problems in children and toddlers range from the city to the global level. The United Nations International Children's Emergency Fund (UNICEF) announced in 2019 that 47 million children worldwide or 6.9% of children under five were undernourished or wasted.[8] In Indonesia, the prevalence of wasted toddlers has reached 7.1% in 2021. Specifically, the rate of wasted toddlers in Jember Regency was the second highest in East Java province in the same year reached 12.8%.[9]

Sucopangepok Village is one of the villages located in Jelbuk Sub-district, Jember Regency. Previous research conducted in Jelbuk Subdistrict showed that the number of toddlers with decreased nutritional status and chronic infections was 67.7% but could not show the correlation between the two.[10] Similar studies in Padang Pariaman Regency and Sampang Regency have been conducted among elementary school students aged over 5 years.[5,11] Since there are limited studies in toddlers, this study aimed to explore the correlation between intestinal protozoa infection and the nutritional status among toddlers aged 12–59 months in Sucopangepok village, Jelbuk sub-district, Jember Regency, East Java, Indonesia.

Methods

This descriptive and analytic observational study with a cross-sectional approach design was conducted from October to December 2022 in Sucopangepok Village, Jelbuk District, Jember Regency, Indonesia, including children aged 12–59 months who were divided into two categories, namely 12–23 months and 24–59 months. This study used non-probability sampling data collection techniques, namely consecutive sampling. A sample size of this study was determined using the Lemeshow formula.[12] Based on the calculation, the minimum sample size needed to represent the entire population of children under five in Sucopangepok Village was 45 children.

After consent from the mothers, data on body weight, height, or length were collected and feces samples were taken to be examined at the Parasitology Laboratory of the Faculty of Medicine, University of Jember. The exclusion criteria were congenital abnormalities, death or dropping out based on parental decisions during the research process, and a history of chronic illness. The ethical clearance of this research has been approved by the Research Ethics Commission of Faculty of Medicine, Universitas Jember no. 1.662/H25.1.11/KE/2022.

Nutritional status assessment used the conversion of body weight and body length or height values in the form of a z-score, calculated using the WHO Anthro Calculator application. The z-score value was read in the weight for length proportion.[13]

Preparation of fecal samples used the sedimentation method. One-gram of feces was placed in a centrifuge tube with distilled water added until evenly distributed. Centrifugation was carried out at 2,000 rpm for 3–5 minutes until the supernatant and sedimentation were separated, then the supernatant was discarded to separate the sedimentation. This step was repeated up to three times. The sedimentation was further prepared for direct smear and modified Ziehl-Nelson staining.

As for direct smear, 10 microliter of sediment was taken and placed on a labeled glass slide. The sample was given a droplet of saline, lugol, or eosin covered with an object glass cover, and immediately observed through a microscope with an objective lens magnification of 10X, 40X, and 100X, to identify all species of intestinal protozoa (except acid-resistant species such as *Cryptosporidium parvum*). The observation results were then matched with WHO guidelines

As for Ziehl-Nelson staining, 100 micromiliters of the sediment was placed on a labeled glass slide. The sample was then fixed over a Bunsen flame. The dried preparation was then given a droplet of methanol above the painting bath and left for three minutes. After the methanol was dried, the preparation was washed in running water. The preparation was then given a drop of ZN A solution and left for 15 minutes to dry then washed again with running water. Furthermore, the preparation was then given a droplet of ZN B for three seconds and then washed again with running water. In the last coloring stage, the preparation was given a drop of ZN C solution for 30 seconds and then washed again with running water, and waited to dry. After drying, the preparation

was then observed through a microscope with an objective lens magnification of 100X. The observation results were then matched with WHO guidelines. This modified ZN staining aims to identify *Cryptosporidium parvum* species.

Bivariate analysis was performed to determine the correlation between intestinal protozoa infection and nutritional status using Cramer's V coefficient test. The p-value less than 0.05 was considered statistically significant. This test also showed a correlation coefficient (r) value which showed the strength and direction of the correlation that was conducted using the Statistical Package for Social Science (SPSS) version 25.0.

Table 1 Sociodemographic Characteristics of the Toddlers Aged 12–59 Months and Parents in Sucopangepok Village, Jelbuk District, Jember Regency

Characteristics	Distribution (n)	Frequency (%)
Age (Months)		
12–23	12	26.7
24–59	33	73.3
Address (Sub-village)		
Krajan Timur	8	17.8
Krajan Barat	4	8.9
Gujuran Barat	9	20
Pangepok	7	15.6
Cangkring	2	4.4
Pakel	9	20
Tenap	5	11.1
Lengkong	1	2.2
Education level		
Mother		
Not completed elementary school	8	17.8
Elementary School	15	33.3
Junior High School	14	31.1
Senior High School	7	15.6
College	1	2.2
Father		
Not completed elementary school	11	24.4
Elementary school	14	31.1
Junior high school	11	24.4
Senior high school	8	17.8
College	1	2.2
Occupation		
Mother		
Unemployed/housewive	35	77.8
Farmer	9	20.1
Shopkeeper	1	2.2
Father		
Farm laborer	3	6.7
Farmer	35	77.8
Private worker	1	2.2
Self-employed worker	4	8.9
Driver	1	2.2
Construction worker	1	2.2
Family Income		
High	6	13.3
Low	39	86.7
Total	45	100

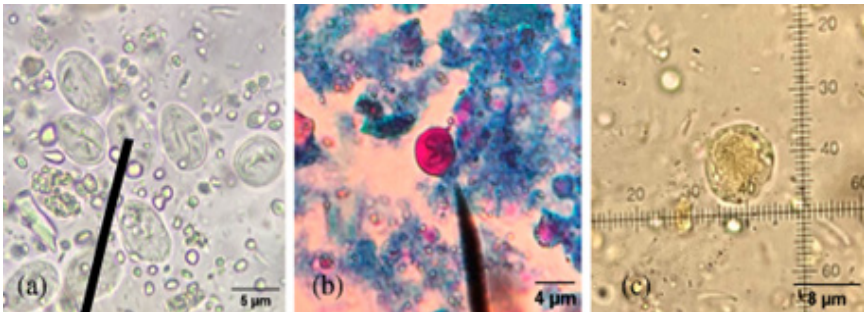

Figure 1 Results of Morphological Observations of Intestinal Protozoa

Note: (a) Giardia lamblia Cyst (Direct smear method 1000X); (b) Cryptosporidium parvum Oocyst (Modified ZN Staining 1000X) ; (c) Vacuolar form of Blastocystis hominis (Direct smear method 1000X)

Results

In total, 45 children were included. The education of the mothers were mostly elementary school graduates, and were unemployed or housewives (77.8%); whereas the majority of the fathers were farmers with monthly income lower than the minimum wage of Jember Regency) as shown in Table 1.

Stool sample examination results showed that 15.6% of respondents were infected with three intestinal protozoa (Table 2), with the highest percentage by *G. lamblia* (6.7%) and *C. parvum* (6.7%) as depicted in Figure 1.

There were toddlers with nutritional status defined as wasted (15.6%) and severely wested (2.2%) as shown in Table 3. There was no correlation between intestinal protozoa infection and nutritional status (p-value =0.441) with a correlation coefficient (r) of 0.191 as depicted in Table 4.

Discussion

Children aged 12–59 months in Sucopangepok village in this study have been infected with intestinal protozoa (15.6%), slightly smaller than the findings in Ethiopia (20.4%).[14] This result might be influenced by several factors such as poor personal hygiene and sanitation and transmission through livestock.[15]

Cryptosporidium parvum is one of the intestinal protozoa species found to infect the children in this study (6.7%). *C. parvum* is the most common species infecting children under five,[16] due to poor sanitation and transmission from livestock or vice versa.[17] Poor sanitation, including the use of untreated water and indiscriminate defecation, is the major risk factor as shown in our study. The residents in Sucopangepok Village have livestock near their homes. Interestingly, these children infected with *C. parvum* have no clinical symptoms, suggesting the opportunistic nature of this species. In contrast, a study in Mozambique has shown that 12.1% of children with *C. parvum* infection showed clinical manifestations of diarrhea.[16] Infected and asymptomatic children who are not treated immediately can be the source of infection in the community.[17] This can happen when the child has poor sanitation such as indiscriminate defecation. Oocysts that come out with feces have good resistance in the environment and infect the water sources used by the residents. Livestock such as cattle might be also affected. *C. parvum* oocysts can enter cattle that drink water or eat grass contaminated with *C. parvum*, and

Table 2 Distribution and Frequency of Intestinal Protozoa Infections among Toddlers Aged 12–59 Months in Sucopangepok Village, Jelbuk District, Jember Regency

Infection Status	Distribution (n)	Frequency (%)	Total n (%)
Positive			
Giardia lamblia	3	6.7	7 (15.6)
Cryptosporidium parvum	3	6.7	
Blastocystis hominis	1	2.2	
Negative	38	84.4	38 (84.4)
Total	45	100	45 (100)

Table 3 Distribution and Frequency of Nutritional Status of Toddlers

Nutritional Status (Weight for length)	Distribution (n)	Frequency (%)
Severely Wasted	1	2.2
Wasted	7	15.6
Normal	29	64.4
Possible Risk of Overweight	5	11.1
Overweight	3	6.7
Obesity	0	0
Total	45	100

Table 4 Cramer's V Test Results

Infection Status	Nutritional Status (Weight for length)			Total (%)	r	p-value
	Severely wasted & wasted (%)	Normal & Possible Risk of Overweight (%)	Overweight & Obesity (%)			
Positive	2 (4.4)	4 (8.9)	1 (2.2)	7 (15.6)	0.191	0.441
Giardia lamblia	1 (2.2)	2 (4.5)	0 (0)			
Cryptosporidium parvum	0 (0)	2 (4.5)	1 (2.2)			
Blastocystis hominis	1 (2.2)	0 (0)	0 (0)			
Negative	6 (13.3)	30 (66.7)	2 (4.4)	38 (84.4)		
Total (%)	8 (17.8)	34 (75.6)	3 (6.7)	45 (100)		

Note: r=Correlation coefficien

become a reservoir host that can further transmit the disease.[18]

Cryptosporidium parvum infection does not only affect the environment but is also harmful to the children themselves. Due to mucosal disruption, absorption surfaces are reduced, leading to impaired growth.[17] The inflammatory process in the small intestine is increased due to *C. parvum* infection, resulting in a state of malabsorption of various nutrients needed by the child, thus triggering wasted nutrition and aggravating the condition of children who have a low immune system.

Furthermore, *Giardia lamblia* is a species that is also found in this study (6.7%), in line with a study in Mozambique showing that *Giardia lamblia* is the most prevalent infection.[16] Various factors such as the use of untreated water, the habit of not washing hands before eating in children or before cooking (caregivers), and the habit of not washing hands after defecation may play roles. *Giardia lamblia* has good resistance in the environment. *G. lamblia* cysts that remain in the consumed water may stick to the hands and can be further swallowed. Interestingly, all toddlers who are infected with *G. lamblia* in this study show no clinical symptoms, in contrast with study in Mozambique where 12.1% of children experience diarrhea.[16] This infection has three stages, namely asymptomatic, acute, and chronic.[17] *Giardia lamblia* cysts are present in infected individuals although there are asymptomatic. Cysts can be found in fecal samples, and become a source of infection if not treated immediately. Giardiasis can occur when there are >100 cysts, while cysts <10 do not.[19] Acute symptoms of giardiasis may include steatorrhea, abdominal pain, bloating, belching, flatus, and vomiting. Inadequate treatment can trigger the chronic phase of giardiasis. Damage to microvilli due to trophozoite invasion of the small intestinal epithelium and trophozoite multiplication covering the surface of the intestinal lumen causes impaired nutrient absorption, which can lead to impaired nutritional status in the form of waste.

Blastocystis hominis is another species found in small numbers in this study with no clinical manifestations. Interestingly, a study in Surabaya showed children infected with B. hominis who had diarrhea.[4] The majority still assume that *B. hominis* is a commensal intestinal parasite.[18] *B. hominis* infection might be caused by poor personal hygiene, exposure to farm animals, and consuming contaminated food and beverages. This intestinal protozoan is considered pathogenic if the ameboid form is found in five visual fields without

other pathogens in a vacuolar form. This species has an opportunistic nature, when the host is in a state of decreased immune system such as in children with malnutrition. Clinical manifestations may not appear in immunocompetent individuals.[20] This finding also needs to be a concern because *B. hominis* is known to be transmitted through contaminated water (waterborne). Children who are wasted and infected with *B. hominis* infection that is not treated immediately can lead to severe wasted.

This study found a small number of severely wasted children (2.2%). This might occur due to a worsening of the nutritional status of children who were previously wasted. This worsening can be triggered by infections in the intestinal tract. Malnutrition in children under five begins with inflammation and damage to the intestinal tract. This situation encourages the malabsorption of various nutrients needed by children so that children experience malnutrition (wasted).[21] This state of malnutrition can reduce the mucosal immune response so that persistent infections that are not treated immediately in children can exacerbate the degree of severity.[24] Failure to treat malnutrition becomes a problem in the future because it can reduce cognitive capacity, work capacity, immunity, and increase the risk of metabolic disorders.[23]

Interestingly, there are children with overweight status (6.7%) as also found in general in other populations.[24] Furthermore, the findings of this study indicate that there is no correlation between intestinal protozoa infection and nutritional status, opposite to the study in Egypt.[25] This non-correlated result may be due to the fact that intestinal protozoan infection is more prevalent in children with normal nutritional status than undernourished and wasted children. This is in line with a recent study in Asia which showed that 73.1% of toddlers infected with intestinal protozoa had normal nutritional status.[7] Infection in children with normal nutritional status can be triggered by low family income levels, unprotected water sources, consumption of raw water, poor sanitation, caregivers not washing their hands properly (before cooking and after cleaning the child's anus after defecation), and children who do not wash their hands properly (before eating and after defecation).[7] This lack of correlation may also be influenced by other factors affecting nutritional status besides intestinal protozoa infection.

Another factor that may affect the results of this study is the nutritional intake received by children. A research showed that there is a correlation between nutritional intake and children's nutritional status.[26] Nutritional intake also depends on other factors such as food security and parenting. The majority of fathers of children work as farmers, ensuring the availability of family food security even though the majority have low incomes. The number of mothers who do not work provides more time to provide good care for children. These things can affect the nutritional status of children in this study.

The limitation of this study is that it only correlates intestinal protozoan infection with the nutritional status of toddlers, without correlating with other nutritional status factors so it cannot compare the most influential factors. The factors may include socioeconomics, parenting, food security, and nutritional intake.

In conclusion, there is no correlation between intestinal protozoan infection and nutritional status of children aged 12–59 months in Sucopangepok Village, Jelbuk Sub-district. Jelbuk. However, a comprehensive cooperation between the government and the community needs to be improved to deal with nutritional problems and to prevent intestinal protozoa infection. Clean and Healthy lifestyle need to be encouraged thoroughly.

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