



Prevalence and risk factors of intestinal protozoan infection among child students with disabilities in Bantul District, Yogyakarta Special Region, Indonesia

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ABSTRACT

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Children with disabilities are excluded from many aspects of life. Unfortunately, they have an increased risk of infection from many kinds of pathogens including intestinal protozoan. This study aimed to determine the prevalence of intestinal protozoan infections and to evaluate the associated factors among children with disabilities in Bantul District, Yogyakarta Special Region, Indonesia. A cross-sectional study was conducted at school with special needs between June-December, 2019. A total of 150 participants were recruited through simple random sampling. Stool samples were examined microscopically by formalin-ether concentration and Ziehl-Neelsen staining technique. Age was analyzed using the Mann-Whitney tests, while the other variables used chi-square tests. Multivariable logistic regression was conducted to identify factors associated with intestinal protozoan infections. The adjusted prevalence ratio with a 95% confidence interval at a 5% level of significance was used to measure the strength of association. Overall, there were 15 children infected by intestinal protozoan among 130 subjects with mean age of participants of 9.83 ± 3.1 years. The intestinal protozoan species were *Entamoeba histolytica* 7 (5.38%), *Giardia lamblia* 4 (3.08%), *Blastocystis hominis* 7 (5.38%) and *Iodamoeba butschlii* 1 (0.77%). Prevalence of intestinal protozoan infection among children with disabilities in Bantul District, Yogyakarta, Special Region was 11.54%. There were no significant correlations between the risk factors and intestinal protozoan infection among children with disabilities ($p > 0.05$).

ABSTRAK

Penyandang disabilitas memiliki kesempatan terbatas dalam berbagai aspek. Anak penyandang disabilitas berisiko terinfeksi berbagai patogen termasuk infeksi protozoan usus. Tujuan penelitian ini adalah untuk mengetahui prevalensi infeksi protozoan usus pada anak penyandang disabilitas dan faktor risiko yang berpengaruh terhadap infeksi protozoa usus di Kabupaten Bantul, Provinsi DIY. Penelitian dilakukan di Sekolah Luar Biasa, dengan metode potong lintang pada bulan Juni-Desember, 2019. Sejumlah 150 anak penyandang disabilitas direkrut dengan metode *simple random sampling*. Sampel tinja diperiksa secara mikroskopis dengan metode terkonsentrasi formalin-eter dan *Ziehl-neelsen* untuk mendeteksi protozoan usus. Variabel usia dianalisis dengan uji Mann-Whitney sedangkan variabel jenis kelamin, status sosial, status ekonomi, tingkat pendidikan orang tua, perilaku, kebersihan diri, dan lingkungan dianalisis dengan *chi square*. Analisis regresi logistik digunakan untuk mengidentifikasi kekuatan faktor risiko terhadap infeksi protozoan usus. *Prevalence ratio* ditetapkan dengan tingkat kepercayaan 95% pada level signifikansi 5%. Lima belas anak (11,54%) dari keseluruhan 130 subyek terinfeksi protozoan usus dengan rerata usia anak adalah $9,83 \pm 3,1$. Spesies protozoa usus diantaranya *Entamoeba histolytica* 7 (5,38%), *Giardia lamblia* 4 (3,08%), *Blastocystis hominis* 7 (5,38%) dan *Iodamoeba butschlii* 1 (0,77%). Tidak terdapat faktor risiko yang signifikan terhadap infeksi protozoan usus ($p > 0,05$). Prevalensi infeksi protozoan usus pada anak penyandang disabilitas usia sekolah di Kabupaten Bantul, Provinsi DIY adalah 11,54%. Tidak terdapat faktor risiko signifikan terhadap infeksi protozoan usus di antara anak penyandang disabilitas ($p > 0,05$).

Keywords:

intestinal protozoa;
formalin-ether
concentration technique;
Ziehl-Neelsen staining
technique;
risk factors;
children with disabilities;

INTRODUCTION

Intestinal parasite infections continue to be an important worldwide public health problem. Intestinal protozoan infection is caused by one or more of the pathogen parasites, such as *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Cryptosporidium parvum*. Intestinal protozoan infections are also caused by nonpathogenic parasites such as *Blastocystis hominis*, *Iodamoeba butschlii*, *Entamoeba coli*.¹ Clinical manifestation of intestinal protozoan infection varies such as chronic diarrhea, anemia, malabsorption, malnutrition, chronic intestinal inflammation, failure to thrive, as well as physical and mental disabilities.²⁻³ There are about 3.5 billion people in the world infected with intestinal protozoan. About 450 million among them become sick and about 16 million die every year.⁴⁻⁵ Intestinal protozoan are widespread in the tropical and subtropical countries.⁶ Indonesia is one of the tropical countries that has become endemic for intestinal protozoan infections. The Special Region of Yogyakarta reported a 24.2% of intestinal protozoan infection in primary school children.⁵ Disability is a term covering weaknesses, including physical limitation or poor body structure, with limitations in daily activities and social participation.⁷ People with disabilities often have difficulty in understanding information and communication with others.⁸ This could affect their understanding about the importance of personal hygiene and create difficulty to access safe water.⁹ Lack of personal hygiene such as rarely washing hand, cutting finger nails, or not using proper toilet put children with disabilities in risk to suffer from many infections including protozoan infection.^{2,3,10} Other habits such as eating raw vegetables, drinking non-boiled water, and using river as water source could increase the protozoan infection.^{3,11} The other risk

factors that might be contributed are parent's low income and or education level.¹¹⁻¹²

MATERIALS AND METHODS

Subjects

This cross sectional study was conducted at 20 schools with special need in the Bantul District, Yogyakarta Special Region, Indonesia. Subjects of study and stool samples were obtained from June until December 2019 and then were examined at the Department of Parasitology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta.

The minimum sample size was calculated using an estimated proportion of the population with the following formula.¹³

with an expected prevalence of the true prevalence, $\pm 8\%$ ($d=0.08$) with a 95% confidence level ($z_{1-\alpha/2}=1.96$) and proportion from the previous study was 24%. From this formula, the minimum sample size of the study was 109 samples.

Protocol of study

Subjects were selected using simple random sampling. Verbal and written research information and a request for consent were provided to the parents. The inclusions criteria were children 5 – 17 years old within the study area at the time of sampling. Subjects were excluded if they had or were receiving treatment for intestinal parasites, such as metronidazole, within two weeks before the interview. Exclusion of these children was important to avoid misinterpretation during stool specimen examination.

Risk factors of intestinal protozoan infection were obtained by interview with the parents of the subjects using questionnaire that has been tested for validity and reliability prior to field

work. Age was calculated based on the provided date of birth and the date of interview. Sex was recorded as male or female. Type of disability was detected from school data. Degree of disability for understanding and communicating was assessed by questionnaire. Social status, degree of parent's education, economic status, and hygiene habit were assessed by questionnaire. The interview was done by the principal investigator.

To ensure maximum co-operation from study subjects, we informed the teachers and parents of each school about the study and the flow of collecting data from interview and stool specimen collection. We provided parents with a package for stool collection: an A5-sized sheet of oil paper to use as the base during defecation, a pre-labeled 60 mL screw-cap stool container; personal protective equipment (a pair of hand gloves and surgical mask) and instruction for how to collect the stool samples and to avoid contamination with sand or other material, details of the amount of stool specimen (about 40 mL), how to confirm that the container was sealed correctly, how to dispose of all equipment except the container with plastic wrap after collection, and the appropriate time to return the container. Parents were asked to return the containers with fresh stool specimens to the school in the morning.

All specimens were brought to the Laboratory of Parasitology for identification then preserved in 10% formalin. Intestinal protozoan was identified from stool specimens by light microscope examination using formalin-ether sedimentation method with iodine according to the World Health Organization standard procedures.¹⁴

Species identified were confirmed directly by a senior laboratory technician. Modified Ziehl-Neelsen staining provided identification for acid-fast protozoan including cysts from many species of *Cryptosporidium spp.*

Statistical analysis

All data analysis was performed using SPSS® version 25 (IBM Corp., Chicago). The prevalence and type of intestinal protozoan infection were obtained from stool examination results using descriptive statistics. Questionnaire data without available stool specimens were excluded. Association between age and intestinal protozoan infection was measured by Mann-Whitney tests. Association between the other risk factor with intestinal protozoan infection were analyzed using chi square tests. A p value <0.05 were considered statistically significant. Relative risk between the risk factors with intestinal protozoan infection was estimated as a prevalence ratio with 95% confidence intervals. Protocol of the study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta with certificate No. KE/FK/1305/EC/2019.

RESULTS

There was a total of 150 students from 20 school with special need enlisted, but 20 students did not submit stool specimens and was not analyzed. The mean age of participants 9.83 ± 3.1 years and ranged from 5 to 17 years old. The baseline characteristic of the subject was listed in TABLE 1.

TABLE 1. Baseline characteristic of subjects

Characteristic	Subjects (n=130)
Age [year (mean ± SD)]	5-17 (9.83 ± 3.1)
Sex [n (%)]	
• Male	74 (56.9)
• Female	56 (43.1)
Type of disability [n (%)]	
• Mental	96 (73.8)
• Physic	34 (26.2)
Degree of disability [n (%)]	
• Mild	69 (53.1)
• Moderate	61 (46.9)
Hand washing after latrine [n (%)]	
• Always	88 (67.69)
• Rare	42 (32.31)
Hand washing before food [n (%)]	
• Always	86 (66.15)
• Rare	44 (33.85)
Fingernail cutting [n (%)]	
• Always	98 (75.38)
• Rare	32 (24.62)
Eating raw vegetables [n (%)]	
• Always	118 (90.77)
• Rare	12 (9.23)
Medical checked up [n (%)]	
• Always	82 (63.08)
• Rare	48 (36.92)
Paternal education [n (%)]	
• Low	57 (43.85)
• High	73 (56.15)
Maternal education [n (%)]	
• Low	54 (41.54)
• High	76 (58.46)
Parents income [n (%)]	
• Low	83 (63.85)
• High	47 (36.15)
Source of water [n (%)]	
• Well	112 (86.15)
• Faucet	18 (13.85)

TABLE 1 Baseline characteristic of subjects (continued).

Characteristic	Subjects (n=130)
Family size [n (%)]	
• Small	39 (30)
• Big	91 (70)
Toilet [n (%)]	
• Opened	17 (13.08)
• Closed	113 (86.92)
Near to livestock [n (%)]	
• Yes	60 (46.15)
• No	70 (53.85)
Water contact activity [n (%)]	
• Yes	80 (61.54)
• No	50 (38.46)
Caregiver [n (%)]	
• Parent	120 (92.31)
• Guardian	10 (7.69)
Drinking water source [n (%)]	
• Boiled water	115 (88.46)
• Raw water	15 (11.54)

Among 130 participants, 15 students (11.54%) were infected by single and multiple intestinal protozoan which are listed in TABLE 2. Pathogenic intestinal protozoan were more prevalent than nonpathogenic intestinal protozoan (TABLE 3). The most prevalent protozoan was *E. histolytica* and *B. hominis* (TABLE

3). Based on the light microscopic observations, all the intestinal protozoan were identified as cyst form. No significantly correlation between all the variables investigated with intestinal protozoan infection was observed (TABLE 4-6).

TABLE 2. Prevalence and type of intestinal protozoan infection in disabled children

Intestinal protozoan	Total (N=130)
Single [n (%)]	
• <i>E. histolytica</i>	4 (3.07)
• <i>G. lamblia</i>	3 (2.30)
• <i>B. hominis</i>	5 (3.85)
Multiple [n (%)]	
• <i>E. histolytica</i> + <i>G. lamblia</i> + <i>I. butschlii</i>	1 (0.77)
• <i>E. histolytica</i> + <i>B. hominis</i>	2 (1.54)
Total	15 (11.53)

N=number of examined, n=number of infected

TABLE 3. The type of intestinal protozoan species based on the pathogenicity and stool consistence in disabled children

Protozoan species	Total
Pathogenics [n (%)]	
• <i>E. histolytica</i>	7 (5.38)
• <i>G. lamblia</i>	4 (3.08)
Nonpathogenics [n (%)]	
• <i>B. hominis</i>	7 (5.38)
• <i>I. butschlii</i>	1 (0.77)
Total	19 (14.62)

n=number of infected

TABLE 4. Association between personal risk factor and intestinal protozoan infection in disabled children

Category	Infected	Not infected	p	PR (95%CI)	Expβ
Sex [n (%)]					
• Male	8 (6.15)	66 (50.77)	0.77	0.87 (0.33-2.24)	-
• Female	7 (5.38)	49 (37.69)			
Type of disability [n (%)]					
• Mental	9 (6.92)	87 (66.92)	0.22*	0.53 (0.20-1.38)	2.24
• Physic	6 (4.62)	28 (21.54)			
Degree of disability [n (%)]					
• Mild	7 (5.38)	62 (47.69)	0.60	1.29 (0.50-3.37)	-
• Moderate	8 (6.15)	53 (40.77)			
Hand washing after latrine [n (%)]					
• Always	9 (6.92)	79 (60.77)	0.56*	1.40 (0.53-3.67)	-
• Rare	6 (4.62)	36 (27.69)			
Hand washing before food [n (%)]					
• Always	11 (8.46)	75 (57.69)	0.53	0.71 (0.24-2.10)	-
• Rare	4 (3.08)	40 (30.77)			
Fingernail cutting [n (%)]					
• Always	12 (9.23)	86 (66.15)	1.00*	0.77 (0.23-2.54)	-
• Rare	3 (2.31)	29 (22.31)			
Eating raw vegetables [n (%)]					
• Always	15 (11.54)	103 (79.23)	0.36*	0	-
• Rare	0 (0)	12 (9.23)			
Medical checked up [n (%)]					
• Always	10 (7.69)	72 (55.38)	0.76	0.85 (0.31-2.35)	-
• Rare	5 (3.85)	43(33.08)			

* Fisher exact test; PR=prevalence ratio; Expβ= odds ratio

TABLE 5. The association between socio-demography characteristic with intestinal protozoan infection in disabled children

Category	Infected	Not infected	p	PR (95% CI)	Exp β
Paternal education [n (%)]					
• Low	9 (6.92)	48 (36.92)	0.18	1.92 (0.73-5.08)	0.45
• High	6 (4.62)	67 (51.54)			
Maternal education [n (%)]					
• Low	7 (5.38)	47 (36.15)	0.67	1.23 (0.48-3.19)	-
• High	8 (6.15)	68 (52.30)			
Parents income [n (%)]					
• Low	10 (7.69)	73 (56.15)	0.81	1.13 (0.41-3.12)	-
• High	5 (3.85)	42 (32.31)			

PR=prevalence ratio; Exp β = odds ratio

TABLE 6. The association between environmental risk factors with intestinal protozoan infection in disabled children

Category	Infected	Not infected	p	PR (95% CI)
Source of water [n (%)]				
• Well	13 (10.00)	99 (76.15)	1.00	1.05 (0.26-4.25)
• Faucet	2 (1.54)	16 (13.9)		
Family size [n (%)]				
• Small	6 (4.62)	33 (12.1)	0.27*	0.64 (0.25-1.68)
• Big	9 (6.92)	82 (63.08)		
Toilet [n (%)]				
• Opened	3 (2.31)	14 (10.77)	0.41*	1.66 (0.52-5.29)
• Closed	12 (9.23)	101 (77.69)		
Near to livestock [n (%)]				
• Yes	6 (4.62)	54 (41.54)	0.91	1.06 (0.39-2.91)
• No	9 (6.92)	61 (46.92)		
Water contact activity [n (%)]				
• Yes	8 (6.15)	72 (55.38)	0.49	1.40 (0.54-3.62)
• No	7 (5.38)	43 (33.08)		
Caregiver [n (%)]				
• Parent	13 (10.00)	107 (82.31)	0.32*	1.85 (0.48-7.06)
• Guardian	2 (1.54)	8 (6.15)		
Drinking water source [n (%)]				
• Boiled water	12 (9.23)	103 (79.23)	0.38*	1.92 (0.61-6.02)
• Raw water	3 (2.31)	12 (9.23)		

* Fisher exact test; PR=prevalence ratio

DISCUSSION

In this study the prevalence of intestinal protozoan infection among children with disabilities in Bantul District, Yogyakarta Special Region was 11.54%. The prevalence of intestinal protozoan infection in other countries is varied. A study among children with mental disabilities in Ethiopia reported about 23.1% intestinal protozoan infection,³ whereas in Iran about 24%.¹⁵

No significantly correlation between age and intestinal protozoan infection was reported in this study ($p>0.05$). This result was parallel with previous studies by Sianturi *et al.*¹⁶ and Meskin *et al.*¹⁷ reporting that intestinal protozoan infection could affect all ages of children. No significantly correlation between sex intestinal protozoan infection in children with disabilities was also reported. This result was parallel with previous study by Shehata and Hassanein,⁴ and Bahmani *et al.*¹¹ that reported out children whether boys or girls have a similar risk of becoming infected by intestinal protozoan. This condition may be due to both sexes are like to play around with dirt.¹¹

No significantly correlation between degree of disability with intestinal protozoan infection was observed ($p>0.05$). In this study degree of disability only focused on intelligence aspect (how to understand and communication). Other aspects in assessing degree of disability are not assessed when interviewing the parents.¹⁸ In previous study, no association was found between degree of disability and intestinal protozoan infections.^{4,15,17} In this study, type of disability also had no correlation with intestinal protozoan infection ($p>0.05$). Type of disability is divided into three i.e. physical disability, mental disability and physic-mental disability.¹⁹ Mental disability refers to difficulty in understanding anything, making it is difficult for the person to understand

about good hygiene behavior.^{4,8} Physical disability involves a difficulty in activities and moving around, making it difficult for the person to access clean water.⁹ No association was found between type of disability and intestinal protozoan infections in the previous study.^{3,4,15,17} Because the previous study only took one type of disability i.e. mental disability.^{3,4,15,17}

Social status of children with disabilities was observed by the source of bathing water and the source of drinking water. In this study, both of them had no correlation with intestinal protozoan infection ($p>0.05$). Well water could be one way to transmit intestinal protozoan from animals or humans.¹¹ This was consistent to a previous study conducted by Shrestha *et al.*²⁰ Other study also showed that drinking untreated water has a correlation with intestinal protozoan infection in school children.^{21,22} The differences of this risk factors could be caused by the low prevalence of intestinal protozoan infection in the children with disabilities at Bantul District, Special Region of Yogyakarta (TABLE 5).

In this study, parent's income was obtained by calculating father's and mother's income. No significantly correlation between parent's income with intestinal protozoan infection was observed ($p>0.05$). This finding was consistent with previous study by Fentahun *et al.*³ In addition, level of parent's education of children with disabilities also had no correlation with intestinal protozoan infection ($p>0.05$). This finding was also consistent with previous studies by Meskin *et al.*¹⁷ and Alemu *et al.*²³ The similarity may be due to most of the parent's education in Bantul District was relatively high. It is assumed that parents with high level education would be more aware about possible sources of transmission of disease.⁶

Caregiver for children with

disabilities had no correlation with intestinal protozoan infection ($p>0.05$) in this study. This was consistent to a previous study conducted by Adams *et al.*²⁴ Most of the children with disabilities in Bantul District were assisted by parents besides the caregiver. Hygiene habits consist of washing hands activity, eating raw vegetables, fingernail cutting, and regular medical checkups. All of these habits had no correlation with intestinal protozoan infection ($p>0.05$). This was consistent with the previous study.^{3,11,23} Research has shown that washing hands activity and fingernail cutting have a correlation with intestinal protozoan infection in children.¹²

Eating raw vegetables also had no correlation with intestinal protozoan infection ($p>0.05$), which was consistent with the previous study that conducted by Fentahun *et al.*³ Raw vegetables could contain both soil and water from the earth. Washing vegetables with contaminated water could become a source of transmission for intestinal protozoan.¹⁵ All of the infected subjects had been eating raw vegetables. There are two form of stool consistence in this study i.e. watery and solid consistence. Watery stool consistence was found in 48 subjects (36.92%), which 12 of them (25%) were infected by intestinal protozoa. While solid stool consistence was found in 82 subjects (63.08%) and 3 of them (3.66%) were infected by intestinal protozoan. This means that watery stool more often indicated intestinal protozoa in children with disabilities. These results are in line with previous studies by Berhe *et al.*²² i.e. pathogenic and nonpathogenic intestinal protozoan can be found in liquid faeces.

Medical checkups periodically had no correlation with intestinal protozoan infection ($p>0.05$), which was contrary with the previous study by Fentahun *et al.*³ Medical checkups periodically could upgrade parent's awareness to practice hygienic habits in their daily activity.

Family size had no correlation with intestinal protozoan infection ($p>0.05$). This study was consistent to a previous study by Shrestha *et al.*²⁰ that found both small and big sized families can maintain cleanliness at home, and this could prevent the transmission of intestinal protozoan. Houses near to livestock had no correlation with intestinal protozoan infection ($p>0.05$). This result was in line with a previous study conducted by Sarkari *et al.*⁶ This similarity indicated that there was no contamination in the family's food by the stool of animals in homes of the children with disabilities at Bantul District.

The use of a toilet had no correlation with intestinal protozoan infection ($p>0.05$). This result is different from previous study by Fentahun *et al.*³ i.e. defecation habit outside the house has more risk to get intestinal protozoan infection compared to defecation inside the house.³ Contact history with public water had no correlation with intestinal protozoan infection ($p>0.05$). This result is in line with studies by Alemu *et al.*²³ and Sitotaw *et al.*²⁵ that showed no correlation between contact history with public water and intestinal protozoan infection. Meanwhile, swimming pools could be contaminated by cysts of intestinal protozoan from the contaminated swimmer.²⁶

Multivariable logistic regression showed that mental disability was the variable with the highest risk for intestinal protozoan infection with Exp^{β} 2.24 (TABLE 4 and 5). This result means person with mental disability were 2.24 more at risk to get infected by intestinal protozoan than physical disability with clinically significant. However, it was not statistically significant ($p>0.05$). There was no information concerning the prevalence of intestinal protozoan infection before this study in the Bantul District Public Health office. The limitation of this study was that there was only one stool sample examined in

the Laboratory of Parasitology. It should include examining the stool from the person several other times because of the cycle of the intestinal protozoan.

CONCLUSION

Prevalence of intestinal protozoan infection in children with disabilities in Bantul District, Yogyakarta Special Region is 11.54%. Age, sex, type of disability, degree of disability, hygiene habits, level of parent's education, economic status, and social status does not have correlations with intestinal protozoan infection in child students with disabilities at Bantul District. Stool examinations need to be routinely conducted to get more valid information about the prevalence of intestinal protozoan infection.

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