

Distribution of enteric protozoan infection among school children in rural and urban areas in West Indonesia: A comparative cross-sectional school-based study

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Abstract. *Background and aim:* The enteric protozoan infection has a high morbidity impact, especially in the pediatric age group. It remains a health problem in tropical countries such as Indonesia due to the limited availability of epidemiological data. This study aims to determine enteric protozoan infection distribution among school children in urban and rural areas in Jakarta-Bogor, Indonesia. *Methods:* The study was conducted between July and December 2018 involving 812 school children from the Bogor Regency as a rural area and the West Jakarta Administrative City as an urban area. A minimum of 13 students aged 5-10 were sampled through random selection from 30 schools. The enteric protozoa are detected from the observed feces using the direct microscopic examination and the data were processed using the SPSS version 20. *Results:* The overall prevalence of enteric protozoan infection is 21.8%, with 32.7% and 12.5% in rural and urban areas. It is more dominant in children aged 7-10 at 12.9% compared to those between 5 and 6 at 8.9%. The three dominant protozoa detected in this study are *Blastocystis hominis*, *Giardia intestinalis*, and *Entamoeba coli*. *Entamoeba histolytica* and *Iodamoeba buetschlii* are not identified in the urban area. *Conclusions:* The prevalence of enteric protozoan infection in children between the ages of 5 and 10 in rural areas is higher than in urban areas. Therefore, interventions such as mass examination of feces followed by antiprotozoal treatment and health education, as well as improvement of sanitation facilities are needed, especially for children living in rural areas. (www.actabiomedica.it)

Key words: *Blastocystis hominis*, *Entamoeba coli*, enteric protozoa, *Giardia intestinalis*, rural, urban

Introduction

Enteric or intestinal protozoa are transmitted through the consumption of water and food contaminated with oocyst or cyst-stage protozoa, waste products not processed according to procedures, as well as the poor maintenance of domestic animals and livestock. The infection can be identified in all countries and is closely related to low socioeconomic conditions, low education and knowledge levels, population density, poor environmental sanitation, and lack of access to clean water (1). Pathogenic enteric protozoa are

associated with clinical manifestations of diarrhea that can be severe when the immunocompromised group is infected. The chronic or recurrent type of this infection, especially in children, can lead to malnutrition, a decrease in the quality of human resources, economic problems in the household, and an increase in health financing (2).

The enteric protozoan species often discovered in feces and considered important for public health include *Cryptosporidium spp.*, *Giardia intestinalis*, *Entamoeba histolytica*, *Dientamoeba fragilis*, *Cyclospora cayentanensis*, and *Blastocystis spp* (1,3). *Cryptosporidium spp.*,

G. intestinalis, and *E. histolytica* causes water-borne infection (1,4). *Cryptosporidium spp.*, *G. intestinalis*, *E. histolytica*, and *C. cayetanensis* are species that cause food-borne disease (1,5). Meanwhile, *Cryptosporidium spp* (6), *Blastocystis spp* (7), and *G. Intestinalis* (8) have zoonotic potential, thereby increasing the risk of infection in humans. These enteric protozoa can be detected in both clinical samples from individuals experiencing diarrhea and survey results from asymptomatic populations, especially in the pediatric age group (9,10). Indonesia is an archipelagic country with 5 large islands, including Papua, Kalimantan, Sumatra, Sulawesi, Java, and thousands of small islands. This impacts on varying geographical locations, different cultural factors and habits of the community, inequality in development, and the socioeconomics of the population (11). According to The Central Bureau of Statistics, 93.4% and 67.2% of the average household in Jakarta and West Java Provinces have access to adequate drinking water. Furthermore, the average proportions of households that have access to proper sanitation are 89.3% and 59.4% (12). Enteric protozoa, as one of the infectious agents closely related to sanitation, have varying prevalence in the population. The prevalence of enteric protozoa in Indonesia ranges from 10.5% to 40.6% in rural and urban areas (10,13–16). There is limited data on enteric protozoa in the population, especially

among Indonesian children. This study aims to determine the prevalence and species distribution of enteric protozoa in children based on the grouping of urban or rural areas in Jakarta-Bogor, Indonesia.

Materials and Methods

Study area

The study is conducted in Bogor Regency, West Java, as a rural area and West Jakarta Administrative City, Jakarta Provinces as an urban area, as shown in Figure 1. Bogor Regency is one of the several buffer zones of Jakarta Province which is also the capital city of Indonesia. This regency has a variety of regional morphology, lowlands, and highlands. The area is crossed by seven watersheds and has hundreds of irrigation networks and springs. This geographical condition is supported by high rainfall; hence, Bogor Regency is suitable for agriculture (17,18). Agriculture remains the leading sector in Bogor, despite the decrease in the land mass of the area (18,19). Based on this geographical condition, this regency can be categorized as a rural area. West Jakarta Administrative City comprises the urban area category bounded by North Jakarta, South Jakarta, Central Jakarta,

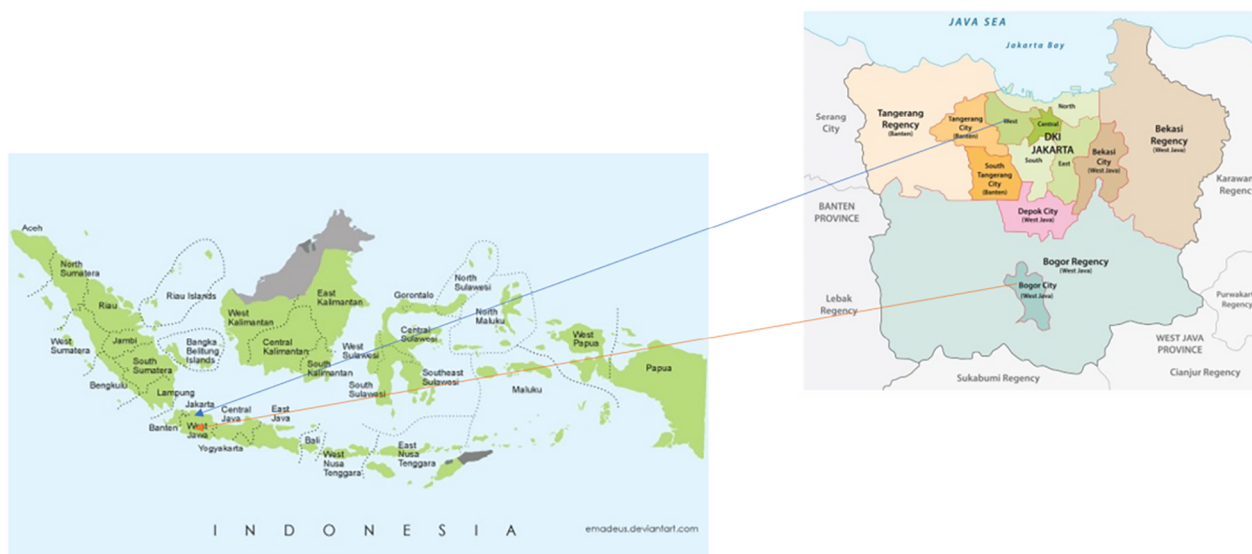


Figure 1. Study locations in rural (green arrows) and urban (red arrows) areas (22).

North Jakarta, and Tangerang. The people's livelihoods are primarily based on trade, industry, and service providers (20). The population density of West Jakarta is 2,434,511 inhabitants which is the highest among other cities in Jakarta Province (21).

Samples

A School-based survey with a cross-sectional design was conducted on kindergarten and elementary school-age children (5 until 10 years old). It was conducted between January and October 2018. Subjects were recruited using two stages of random sampling; first, we randomly selected 30 districts in each rural and urban area. Next stage randomly selected one school

for each district. The minimum sample size required in this study is calculated using the following formula $n=(Z_a^2 \times P \times Q)/d^2$, with a degree of type one error with a predetermined significance level of 5% ($Z_a= 1.96$), the proportion of protozoan infection was 40% (16). It re-determined the absolute precision level of 5% ($d= 0.05$). Based on the calculation, each area's minimum sample size is 396. The eligible population is a minimum of 13 students between the ages of 5 and 10 randomly selected from 30 Kindergartens/Elementary Schools in Bogor Regency and West Jakarta Administrative City as rural and urban areas, respectively. Assuming 50% of participants would submit a sample, we selected 30 students from each school who will be given the fecal container (Figure 2). The inclusion criteria for this

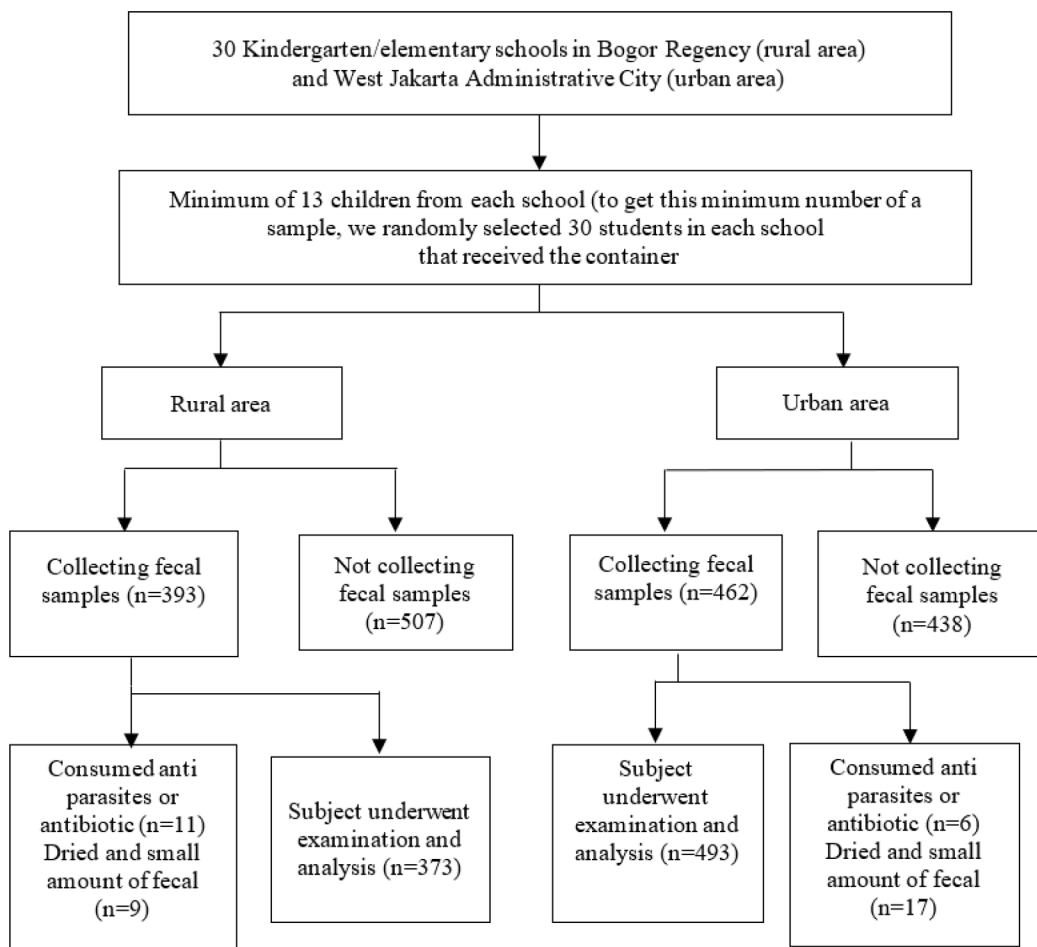


Figure 2. Subjects participation scheme.

study were children between 5- and 10 years old, living in Bogor Regency or West Jakarta Administrative City, allowed by their parents to participate in the study, and returning plastic bottles filled with feces. Exclusion criteria were children with fever and diarrhea and children who received antibiotics, anthelmintic, or antiprotozoal treatment in at least one month. Data on age and gender were obtained from the school database.

Sample collection and parasite fecal examination

This activity was conducted with the help of the principal and teachers from each school. Students were randomly selected, and informed consent was obtained from their parents. Parents were taught how to collect a fecal sample; some children have to be assisted by their parents. A package for fecal collection and written instructions for collecting the fecal and disposing of the waste were distributed to selected students through the parents. The package consists of a clean, dry, and labeled fecal container with a spoon-like cap, fecal catcher (an A4 sized of oil paper), a pair of hand gloves, a zipped plastic bag to wrap the container, and a dark-colored shopping bag. The container using a shopping bag will be returned the next day after being filled with the fecal sample. The labelled were written the code of school, class, age, and name. The collected sample was fixed in 10% formalin and transferred to the Clinical Parasitology Laboratory, Faculty of Medicine, Universitas Indonesia, for examination. The fecal examination is performed by direct technique, wet preparations stained with Lugol's solution, then examined under a microscope in 400x magnification. Each sample was performed by a laboratory technician and confirmed by a medical doctor. A positive sample is infected with enteric protozoa when trophozoites or cysts of enteric protozoan are discovered.

Data analysis

The data obtained are processed and analyzed using SPSS (Statistical Program for Social Science) version 20 software. Before the analysis, the editing, coding, and entry processes are conducted. Furthermore, descriptive, bivariate, and multivariate analyses are performed. A descriptive study is conducted to determine

the distribution and frequency of the variables. Bivariate analysis is conducted using the Chi-Square test to determine the relationship between the prevalence of enteric protozoan infection with education level and gender in each area of residence. The process proceeds to the Fisher exact test when the Chi-Square test conditions are not fulfilled. Multivariate analysis is used to determine the most dominant factor in the incidence of enteric protozoan infection. This study has obtained permission and ethics approval from the Health Research Ethics Committee, Faculty of Medicine, the University of Indonesia with No. 0880/UN2.F1/ ETIK/2018.

Results

This study obtained 812 samples, out of which 373 and 439 were from rural and urban areas, respectively. According to the data on age group and education level, most subjects are 7-10 years old and attending elementary school, marking up to 67.9%. Meanwhile, based on gender, the proportion of male samples is more than females, with 53.6% and 46.4%, respectively. The prevalence of school children experiencing single and mixed enteric protozoan infections is 32.7% in rural areas and only 12.5% in urban areas, as shown in Table 1. The proportion of boys infected with enteric protozoa is 12.4%, with 17.2% in rural and 8.4% in urban. Based on age group, enteric protozoan infection is more common among the ages of 7 and 10, with 12.9%. Based on the area of residence in rural, enteric protozoan infection is higher between the ages of 5 and 6, accounting for 17.7%.

The distribution of enteric protozoan infection in children living in rural and urban areas is described in Table 2. The prevalence of enteric protozoan infection in this study is 21.8% (177/812). Six species are found through feces investigation, with *B. hominis* as a single (53.1%) or mixed infection with other protozoa species (13%) being the most prevalent in both regions. Based on the area of residence or the total sample, single infection of enteric protozoan species is more common than mixed. Furthermore, there were 14.7% of mixed infection detected which consist of 23 infected with 2 species, 2 children infected with 3 species, and only one child infected with four species.

Table 1. Prevalence of Enteric Protozoan Infection by Gender, Education Level, and Area of Residence.

| Demographic characteristics | Rural, n (%) | | Total | Urban, n (%) | | Total | Total, n (%) | | Total |
|-------------------------------------|-------------------|-------------------|-------------------|------------------|-------------------|-------------------|-------------------|-------------------|------------------|
| | Positive | Negative | | Positive | Negative | | Positive | Negative | |
| Age group by education level | | | | | | | | | |
| Kindergarten (5-6 years) | 66 (17.7) | 141 (37.8) | 207 (55.5) | 6 (1.4) | 48 (10.9) | 54 (12.3) | 72 (8.9) | 189 (23.3) | 261 (32.1) |
| Elementary school (7-10 years) | 56 (15) | 110 (29.5) | 166 (44.5) | 49 (11.2) | 336 (76.5) | 385 (87.7) | 105 (12.9) | 446 (54.9) | 551 (67.9) |
| Gender | | | | | | | | | |
| Male | 64 (17.2) | 118 (31.6) | 182 (48.8) | 37 (8.4) | 216 (49.2) | 253 (57.6) | 101 (12.4) | 334 (41.1) | 435 (53.6) |
| Female | 58 (15.5) | 133 (35.7) | 191 (51.2) | 18 (4.1) | 168 (38.3) | 186 (42.4) | 76 (9.4) | 301 (37.1) | 377 (46.4) |
| Total | 122 (32.7) | 251 (67.3) | 373 (45.9) | 55 (12.5) | 384 (87.5) | 439 (54.1) | 177 (21.8) | 635 (78.2) | 812 (100) |

Table 2. Prevalence and Distribution of Enteric Protozoa by Area of Residence.

| Enteric protozoan species | Rural (n= 373) | | Urban (n=439) | | Total (n=812) | |
|--|----------------|--------------|---------------|--------------|---------------|-------------|
| | n | % | n | % | n | % |
| Single infection | 103 | 84.4 | 48 | 87.3 | 151 | 85.3 |
| <i>B. hominis</i> | 61 | 49.2 | 34 | 61.8 | 94 | 53.1 |
| <i>G. intestinalis</i> | 18 | 14.8 | 7 | 12.7 | 25 | 14.1 |
| <i>E. coli</i> | 14 | 11.5 | 5 | 9.1 | 19 | 10.7 |
| <i>E. histolytica</i> | 2 | 2.5 | 0 | - | 3 | 1.7 |
| <i>E. nana</i> | 8 | 6.6 | 2 | 3.6 | 10 | 5.6 |
| Multiple infections | 19 | 15.6 | 7 | 12.7 | 26 | 14.7 |
| <i>B. hominis</i> + <i>G. intestinalis</i> | 9 | 7.4 | 3 | 5.5 | 12 | 6.8 |
| <i>B. hominis</i> + <i>E. Coli</i> | 1 | 0.8 | 2 | 3.6 | 3 | 1.7 |
| <i>B. hominis</i> + <i>E. nana</i> | 3 | 2.5 | 1 | 1.8 | 4 | 2.3 |
| <i>B. hominis</i> + <i>E. histolytica</i> | 1 | 0.8 | 0 | - | 1 | 0.6 |
| <i>G. intestinalis</i> + <i>E. coli</i> | 2 | 1.6 | 0 | - | 2 | 1.1 |
| <i>G. intestinalis</i> + <i>E. nana</i> | 1 | 0.8 | 0 | - | 1 | 0.6 |
| <i>B. hominis</i> + <i>E. coli</i> + <i>E. nana</i> | 0 | - | 1 | 1.8 | 1 | 0.6 |
| <i>B. hominis</i> + <i>G. intestinalis</i> + <i>E. nana</i> | 1 | 0.8 | 0 | - | 1 | 0.6 |
| <i>B. hominis</i> + <i>G. intestinalis</i> + <i>E. coli</i> + <i>I. buetschlii</i> | 1 | 0.8 | 0 | - | 1 | 0.6 |
| Total | 122 | 100.0 | 55 | 100.0 | 177 | 100 |

Abbreviations: *B. hominis*= *Blastocystis hominis*; *G. intestinalis*= *Giardia intestinalis*; *E. Coli*= *Entamoeba coli*; *E. nana*= *Endolimax nana*; *E. histolytica*= *Entamoeba histolytica*; *I. buetschlii*= *Iodamoeba buetschlii*

The enteric protozoans not found in the urban area are *E. histolytica* and *Iodamoeba buetschlii*.

Tables 3 and 4 show the relationship between the frequencies of the three most enteric protozoan

species in both areas by age and gender. In this study, *B. hominis* and *G. intestinalis* infections are higher in males than females, but not statistically significant. In rural and urban areas, *G. intestinalis* infection is higher

Table 3. Prevalence of Infection with three Enteric Protozoan Species by Age Group and Gender in The Rural Area.

| Demographic status | n | <i>B. hominis</i> (single or mixed) | | <i>G. intestinalis</i> (single or mixed) | | <i>E. coli</i> (single or mixed) | |
|--------------------|-----|--|------|---|------|-------------------------------------|-------|
| | | Prevalence | p* | Prevalence | p* | Prevalence | p* |
| Age group | | | | | | | |
| Kindergarten | 207 | 39 (10.4%) | 1 | 14 (3.8%) | 0.22 | 9 (2.4%) | 0.213 |
| Elementary school | 166 | 37 (10%) | | 18 (4.8%) | | 9 (2.4%) | |
| Gender | | | | | | | |
| Male | 182 | 45 (12%) | 0.38 | 18 (4.8%) | 0.49 | 4 (1.1%) | 0.2 |
| Female | 191 | 31 (8.3%) | | 14 (3.8%) | | 14 (3.8%) | |

*Chi-Square

Table 4. Prevalence of Infection with three Enteric Protozoan Species by Age Group and Gender in the Urban Area.

| Demographic status | n | <i>B. hominis</i> (single or mixed) | | <i>G. intestinalis</i> (single & mixed) | | <i>E. coli</i> (single or mixed) | |
|--------------------|-----|--|-----|--|-----|-------------------------------------|-----|
| | | Prevalence | p* | Prevalence | p* | Prevalence | p* |
| Age group | | | | | | | |
| Kindergarten | 54 | 6 (1.4%) | 1 | 1 (0.2%) | 1 | 3 (0.7%) | 0.5 |
| Elementary school | 385 | 35 (8%) | | 9 (2.1%) | | 5 (1.1%) | |
| Gender | | | | | | | |
| Male | 253 | 25 (5.7%) | 0.5 | 8 (1.8%) | 0.2 | 5 (1.1%) | 0.4 |
| Female | 186 | 16 (3.6%) | | 2 (0.5%) | | 3 (0.7%) | |

*Chi Square

in elementary school-aged children but not statistically significant. Based on age and gender, there is no statistically significant difference in infection between the three main enteric protozoan species, namely *B. hominis*, *G. intestinalis*, and *E. coli*, in the two areas.

Risk factors for *B. hominis* infection include elementary school education level (OR=1.6; 95% CI=1.09-2.40; p=0.022) and rural area of residence (OR=2.98; 95% CI=1.98-4, 49; p<0.001). Meanwhile, for *G. intestinalis* infection in this study, it is stated to be the rural area of residence (OR=4.02; 95% CI=1.95-8.31; p<0.001), as indicated in Table 5.

Discussion

An enteric protozoan infection affects the digestive tract, especially the intestines. Its prevalence was

identified in children aged between 5 and 10 years living in rural and urban areas, with 32.7% and 12.5%, respectively. An enteric protozoan infection is examined from feces samples using a direct technique, followed by analysis based on age group, gender, and area of residence. A previous study in an urban area discovered intestinal protozoan infection at 37% (15) while the prevalence in a rural area was 20% (10). The study used direct examination techniques to detect enteric protozoa, and the population was also school-age children. A study in Iran discovered this infection in the population of school children at 38.9% and 45.2% in urban and rural areas (23). The higher prevalence could be due to laboratory techniques conducted using the formalin-ether concentration followed by direct examination. Therefore, the sensitivity of the examination is increased. The difference in the laboratory techniques used is due to the absence of a gold standard for the

Table 5. Analysis of Potential Risk Factors for Infection with three Enteric Protozoan Species.

| Protozoa | Gender | | OR | 95%CI | p | Age Group | | OR | 95%CI | p | Area of Residence | | OR | 95%CI | p |
|--|----------|------------|------|-----------|------|------------------|----------------|------|-----------|-------|-------------------|-----------|------|-----------|--------|
| | Male (n) | Female (n) | | | | Kindergarten (n) | Elementary (n) | | | | Rural (n) | Urban (n) | | | |
| <i>B. hominis</i> (single or mixed) | 70 | 47 | 1.17 | 0.79-1.72 | 0.48 | 45 | 72 | 1.61 | 1.09-2.40 | 0.022 | 76 | 41 | 2.98 | 1.98-4.49 | <0.001 |
| <i>Giardia</i> (single or mixed) | 26 | 16 | 1.43 | 0.76-2.71 | 0.34 | 15 | 27 | 1.18 | 0.61-2.27 | 0.73 | 42 | 10 | 4.02 | 1.95-8.31 | <0.001 |
| <i>E. coli</i> (single or mixed) | 9 | 17 | 0.70 | 0.28-1.72 | 0.58 | 12 | 14 | 0.90 | 0.34-2.37 | 1 | 18 | 8 | 2.81 | 1.07-7.39 | 0.05 |

detection of enteric protozoa as well as the availability of funds and experts. The use of laboratory techniques based on molecular detection is proven to be more sensitive and specific, but special funds and infrastructure are needed (24). However, direct microscopic examination of feces has the advantage of detecting more than one parasite species, including worms and protozoa, simultaneously. Data on the prevalence of enteric parasites, especially protozoa in the population, particularly children, vary because they are related to limited services and resources for parasite fecal examination (25), low socioeconomic conditions, poor sanitation, low education level, inadequate access to clean water, poor nutrition, contact with animals (26), eating habits (vegetarian or non-vegetarian, culture eating habit) (27), as well as climatic and geographical conditions (28,29). These conditions are generally discovered in tropical and subtropical countries that have rural areas with favorable geographical conditions for parasite development (30). Furthermore, differences in habits or values adopted in residence also affect the prevalence rate (23). The most susceptible population is school-age children, and enteric protozoan infection is usually chronic and asymptomatic; hence the adverse effects are not directly complained of by parents or patients from the adult group. Chronic infection can cause complications such as anemia, cognitive decline, growth and development disorders, and issues in other organs (31). In this study, the prevalence of intestinal protozoan infection in the Elementary School/equivalent group (7-9 years) is higher in the urban area. Meanwhile, the prevalence of enteric protozoa in rural area is higher in the kindergarten age group. Sebaa et al. (32) identified the highest prevalence of asymptomatic enteric protozoan infection in the 2-10 age group in combined rural and urban areas of South Algeria. In contrast to studies in Iran (23) and Ethiopia (26), the prevalence of enteric protozoa was highest in the older age group of 13 and 15 years. The kindergarten and elementary school age groups are more susceptible to enteric protozoan infection because their immune systems are not fully developed, and they have begun to interact with the environment inside and outside the home. However, there is still a lack of knowledge about the dangers and prevention of infection (33). In older children, infection is associated

with behaviors such as consumption of unwashed vegetables, reluctance to wash hands before eating or after using the toilet, eating raw meat, and dirty nails (26). Differences in infection between the age groups are also influenced by the education level of parents or primary caretakers and habits taught at home (33). Further studies need to be conducted on the knowledge, attitudes, and behaviors related to enteric protozoan infection in the pediatric population and primary caregivers. Based on gender, the prevalence of positive infection with intestinal parasites in both areas is dominated by males. The percentage of enteric protozoan infection in males is 17.2% and 8.4% in rural and urban areas, respectively. These are relatively the same as the study in the rural population in Ghana, with a percentage of 17.9% in males, higher than in females at 11.8% (33). The high prevalence in males could be due to more outdoor activities, such as playing football, cycling, or traditional local games, especially for boys living in rural areas. Furthermore, many livestock are left to move freely, thereby increasing the risk of protozoa transmission. Further analysis found no differences in gender with the prevalence of enteric protozoan infection by *B. hominis*, *G. intestinalis*, and *E. coli* in both study areas. In total, 177 students were found positive for enteric protozoan infection, with 85.3%, 12.9%, 1.2%, and 0.6% infected with one, two, three, and four species, respectively. The prevalence of single infection is higher than that of mixed infection. This figure is similar to a study in a systematic review and meta-analysis study of intestinal parasite infection among children under five in Ethiopia between 2010 and 2023, which showed 28.02% and 0.25% of single and mixed infections, respectively (34). Protozoan infection detected in both areas is *B. hominis* followed by *G. intestinalis*, *E. coli*, and *E. nana*. Meanwhile, *E. histolytica* and *Iodamoeba buetschlii* are only found in rural. The high prevalence rate and the presence of non-pathogenic protozoa can indicate contamination of human or animal feces to food and water (35,36).

The most abundant protozoa detected in this study is *B. hominis*, either as a single (rural 48.2% and urban 61.8%) or mixed infection with other species (rural 13.2% and urban 12.7%). These results are consistent with several studies conducted in rural and urban areas of Indonesia, which stated that *B. hominis* is

the most frequently detected protozoa in the population of school-age children without clinical symptoms (10,13–15,37). This differs from a study in a rural area of Iran, which stated that this protozoan is the 3rd most common parasite after *G. intestinalis* and *E. nana*. It infects a population of children between the ages of 1 and 10 (38). Arikian et al (39) in urban population stated that *B. hominis* is the 2nd most common parasite after *G. intestinalis*. In this study, the risk factors affecting its infection are age group and area of residence. According to a study in Malaysia, the risk factors for parasitic infection related to conditions in rural areas include lack of clean water, inadequate bathing, washing, and toilet facilities, unavailable toilet facilities, open defecation, littering, and low income (2). *G. intestinalis* is a protozoan with the second highest prevalence in rural (single infection 14.8% and mixed infection 11.4%) and urban (single infection 12.7% and mixed infection 5.5%) areas. A contrast infection rate was also reported in the rural areas of Malaysia (Orang Asli) and urban areas of Malaysia at 1.6% and 3.1%, respectively (40). In rural areas with poor sanitation, the absence of household water source management, and poor handwashing practices, the prevalence of giardiasis is relatively high due to contamination of cysts in water used for daily needs (24). According to this study, gender is not a risk factor for *G. intestinalis* infection. This differs from the study conducted by Al-Jawabreh et al. (41) on the population aged 1 to 66, where males are a risk factor for *G. intestinalis* infection. A commensal enteric protozoan identified in the large intestine is *E. coli* at 10.7% as a single infection and 4.0% as a mixed infection with other species in rural and urban areas. This prevalence rate is slightly lower than the results in Nepal of 22.8% (42) and identical to that of Palestina at 8% (41). The prevalence may differ because different tests were used. The prevalence estimate from molecular methods was higher than that from microscopy test (24). A non-pathogenic enteric protozoan in the colon also discovered in rural and urban areas is *E. nana*. There are case reports related to its infection in immunocompetent individuals causing symptoms of urticaria, polyarthrititis, chronic diarrhea, and intestinal inflammation. Scientific evidence is needed to prove the pathogenicity of this protozoan, but caution should be exercised against

the possibility of infectious strains (43). A commensal parasite of the large intestine that is only identified in the rural area (0.6%) and a mixed infection with enteric protozoan species is *I. buetschlii*. This protozoan is commensal and transmitted through contaminated water and food, where symptoms occur when the patient is in the immunocompromised phase. Toychiev et al. (44) stated that the prevalence of *I. buetschlii* in ulcerative colitis patients is significantly higher than in the control sample. These three non-pathogenic protozoa can be indicators of feces contamination and are often co-infected with other organisms (43). Therefore, the presence of these protozoa in the population should be of particular concern. This study also discovered enteric protozoan infection of up to 4 species. Hassen Amer et al. (45) also identified multiple infections of up to 5 species. Multiple enteric protozoan infections are possible due to identical transmission modes. Additionally, unhygienic living conditions, behavior, and food and drink sources increase the risk of being infected with more than one parasite. The presence of one parasitic species in the gastrointestinal tract will cause initial irritation and inflammation (46). Besides rural areas, multiple enteric protozoan infections can also be discovered in slum urban areas. This is related to urbanization activities, the movement of people from rural to urban areas. Excessive migration can increase population density in urban areas. Meanwhile, the facilities and the fulfillment of clean water and sanitation needs cannot be fulfilled properly, creating a mega-slum phenomenon. Furthermore, the tradition of returning home during religious holidays also increases the risk of re-infection from the original environment. There is a decrease in the prevalence of enteric parasitic infection in the community, especially in children, after moving to a flat or apartment. This movement minimizes contact with infection sources (soil and water), but the risk of transmission through unhygienic food or contact with pets is still possible (40). Several studies showed that the prevalence of enteric protozoa is higher in the rural population, especially among breeders, thereby increasing the risk of zoonotic transmission. Furthermore, in rural, livestock is still released in residential areas and landfills that have not been appropriately managed (41). There is a need for additional research to examine the incidence

of protozoa in the general population (not only children) in relation to breeders and the distance of animal cages from residential homes. This study has several limitations in that it employed a school-based sampling method, which may not accurately represent the entire population of school-age children in the regions studied. As a cross-sectional study, it captures data at a single point in time. This limits the ability to establish a causal relationship between infection rates and potential risk factors, as it does not account for temporal changes or the dynamic nature of infections. Furthermore, the study relied on direct microscopic examination of fecal samples for detecting intestinal protozoa. While this method can identify multiple parasite species, it may lack sensitivity compared to more advanced techniques. However, this study provides a comparative analysis between urban and rural populations, highlighting differences in prevalence rates of intestinal protozoan infections. This allows for a better understanding of how environmental and socioeconomic factors influence infection rates among children in different settings. Public health implications of intestinal protozoan infections, particularly their impact on children's health and development, are important. Identifying higher prevalence rates in rural areas underscores the need for targeted interventions such as health education and improved sanitation facilities (47). Given the limited existing epidemiological data on intestinal protozoan infections in Indonesia, this study can fill a critical gap by providing updated prevalence rates and species distribution among school children, which can inform policymakers and healthcare providers.

Conclusion

Enteric protozoan infection is still a significant problem in rural and urban areas in Indonesia. Enteric protozoa *B. hominis*, *G. intestinalis*, and *E. coli* are the most commonly identified species. Therefore, this study provides primary data on the problem of enteric protozoa for appropriate decision-making and intervention at the community level, especially in the pediatric population.

Ethic Approval: Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia No. 0880/UN2.F1/ETIK/2018.

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