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Original Article

Common intestinal parasitic infections in an improved water access, sanitation, and hygiene profile setting in North Jakarta, Indonesia

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Abstract

Intestinal parasitic infections (IPIs), caused by helminths and/or protozoa, continue to be a significant public health concern in Indonesia. Water access, sanitation, and hygiene practices (WASH) are influential factors for IPIs, especially among children. The aim of this study was to investigate the association between WASH and IPIs among school-aged children. A cross-sectional study involving 338 school-age children in an urban slum area in North Jakarta, Indonesia, was conducted using stool specimens subjected to microscopic and real-time polymerase chain reaction (rt-PCR) examination. The children underwent a finger-prick blood test and anthropometric measurements to determine anemia and nutritional status. Parents whose children participated in stool and blood examinations were interviewed using a modified WASH questionnaire. Helminth infections were not found in this study, whereas the overall prevalence of intestinal protozoa parasitic infection (IPPI) was 18.3% and 52.4% by microscopy and rt-PCR, respectively. *Blastocystis* spp. was found to have the highest prevalence (microscopy: 12%; rt-PCR: 48.6%), followed by *Giardia intestinalis* (microscopy: 0.6%; rt-PCR: 6.7%), *Cryptosporidium* spp. (microscopy: 5.1%; rt-PCR: 1.6%), and *Entamoeba histolytica/dispar* (microscopy: 0.6%; rt-PCR: 3.2%). Additionally, *Dientamoeba fragilis* was detected by rt-PCR at 4.1%. Furthermore, the discrepancies between microscopy and rt-PCR were observed in 8.9% (n=28) of the examined specimens. The majority of the respondents had a low-risk category of WASH profile. School children aged 5–10 years old (OR=2.06; 95%CI=1.27–3.33) and those who drank unprocessed cooking water (OR=1.95; 95%CI=1.07–3.57) were significantly associated with IPPI. The present study demonstrated that rt-PCR provides a better understanding of IPI epidemiology and has potential as a monitoring strategy for managing IPIs. Even though this population exhibits an adequate WASH profile and is not directly associated with IPIs, conducting a more in-depth observation of WASH facilities and practices is recommended to ensure a comprehensive assessment of the WASH profile. Additionally, engaging stakeholders in health promotion programs to ensure the sustainability of a good WASH profile and awareness of parasitic infections will be advantageous in achieving optimal urban health.

Keywords: Anemia, children's health, intestinal parasitic infections, nutritional status, WASH

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Introduction

As one of the most persistent global health issues, intestinal parasitic infections (IPIs) are regarded as a major public health problem, particularly in developing countries [1]. It affects approximately 3.5 billion individuals worldwide, with Southeast Asia (SEA) countries being among the most severely impacted [2,3]. Therefore, IPIs, which consist of intestinal helminths and protozoa, are considered significant health issues, especially among children [4].

Intestinal helminths are important causative organisms of IPIs. The helminth infection was predominantly induced by soil-transmitted helminth (STH) species, including hookworms (*Necator americanus* and *Ancylostoma duodenale*), *Ascaris lumbricoides, Trichuris trichiura*, and *Strongyloides stercoralis*. A previous review reported that the prevalence of STH in Indonesian children ranged from 6.6% to 17.1% (16.9 million to 43.5 million cases) [5]. Moreover, another review provides information that the eastern regions of Indonesia exhibit the highest prevalence, reaching 65.8% in 2014 [6]. IPIs commonly affect impoverished communities in tropical regions, including urban slum areas, which is a high-risk condition for parasitic infection [7]. Eliminating IPIs is crucial in urban slum areas because these areas are considered neglected [8]. Among the IPIs, STHs are recognized as one of the 20 neglected tropical diseases (NTDs) [9]. Efforts to eliminate NTDs, including the deworming program, have been implemented by the Ministry of Health (MoH) since 2018 through the Mass Drug Administration (MDA) of albendazole 400 mg every six months (or twice a year), which primarily targets school-age children [10].

Despite receiving limited attention than STHs, intestinal protozoan parasitic infections (IPPIs) are equally important as STHs. The estimated prevalence of IPPIs in Indonesia in 2014 ranged from 4.5% to 34.4% based on microscopic detection [6]. Several species commonly contributed to IPPIs, including *Giardia intestinalis*, *Cryptosporidium* spp., *Entamoeba histolytica*, *E. dispar*, *Dientamoeba fragilis*, and *Blastocystis* spp. Both *Blastocystis* spp. and *Cryptosporidium* spp. have been reported as predominant pathogens responsible for opportunistic infection in individuals with human immunodeficiency virus (HIV) [6,11]. Moreover, a recent review and a study revealed that *G. intestinalis* and *Cryptosporidium* spp. are emerging as significant infections, particularly in regions characterized by low-income and inadequate sanitation [12,13]. In contrast to helminth infections, diagnostic and treatment approaches for protozoan infection remain limited, although IPPIs represent a prominent public health issue in tropical areas [1]. Chronic protozoan infections cause severe health conditions similar to helminth infections, such as malnutrition, stunting, weight loss, and anemia [14].

The impact of IPIs is primarily observed in children. Their underdeveloped immune systems and inherent behaviors, such as the habit of barefoot during outdoor activities, unhygienic fingernails, and ineffective handwashing practices, make them susceptible to infection [15,16]. Additional factors associated with IPIs in children include inadequate maternal education, a lack of piped drinking water, limited latrine availability, and low socioeconomic status (SES) [16,17]. Previous studies have demonstrated that these risk factors, which are part of water access, sanitation, and hygiene (WASH), contribute to IPI incidence [15-17].

The components of WASH encompass various elements that contribute to the improvement of individual and public health. The World Health Organization (WHO) has stated that the explicit inclusion of WASH in the sixth Sustainable Development Goal (SDG) is indicative of its critical significance for human health and well-being [18]. Consequently, the WHO has formulated a standardized WASH survey to evaluate the WASH conditions in all residential settings [19]. Geographical location is one of the multifactorial influences that affect WASH components. In comparison to rural areas, urban areas generally have greater access to clean water and good sanitation, according to previously published studies [20,21]. Nevertheless, disparities continue to exist, and in some developed metropolises, such as Jakarta, the presence of 'slum' areas is prevalent. 'Slum' is defined as an area that is characterized by low-income levels, limited education, and underdevelopment in WASH [8].

Nevertheless, there is a greater possibility of utilizing advanced diagnostic methods in urban areas to detect various pathogens. These diagnostic technologies, such as real-time polymerase chain reaction (rt-PCR), have been widely used for pathogen detection [22]. The rt-PCR method

is capable of detection, amplification, and quantification of the genetic material of specific targets [23]. As a result, it demonstrates increased sensitivity in parasite detection, thereby assisting researchers and healthcare workers in precisely diagnosing IPIs in urban settings [24]. In order to precisely detect specific species using the rt-PCR method, primers-probe pairs need to be specifically designed. This study employed an in-house rt-PCR method to detect the IPIs, which was modified from a previously published study [25]. The in-house rt-PCR panels were specifically designed for detecting intestinal parasite species of interest. Thus, these rt-PCR panels are non-commercial kits. However, these in-house rt-PCR panels could be adapted and used by other researchers interested in detecting targeted species for research purposes.

Some countries have conducted a WASH study in correlation with the IPI, primarily focusing on rural settings and emphasizing WASH to STH, rather than a broader range of gut parasites [26-31]. However, the impact of WASH on slum areas must not be overlooked, as the presence of poor water quality in these areas could potentially act as a risk factor for intestinal protozoa species [32]. Furthermore, studies in epidemiological studies have demonstrated the superiority of rt-PCR over microscopy-based detection in IPI detection [24,25,33,34]. Therefore, the aim of this study was to investigate the prevalence of IPI among children in one of the urban slum areas in North Jakarta using both microscopic and rt-PCR to assess IPIs. Furthermore, this study investigates the relationship between IPI, WASH profile, anemia, and nutritional status. This study is expected to shed light on the advantages of enhancing children's health, especially in the context of urbanization, and to highlight the crucial role of improved WASH profiles in promoting urban health.

Methods

Study area

A cross-sectional study was conducted between February and May 2023 in a primary school located in Kampong Kamal Muara (KKM), an urban slum area in Penjaringan Sub-district, North Jakarta, Indonesia [35]. Kampongs were identified by their informal, dense settlement structures with substandard physical housing and infrastructure. Kampongs represent urban-village communities in cities. KKM has an estimated population of 1,480 households, with fishing being reported as the primary livelihood for its residents. Floods frequently occur in KKM, particularly those caused by seawater from high tide, exposing the communities to litter, pollution, and sewage, which adversely impact their well-being and health [36].

Study population

The study population was comprised of students from first to sixth grades with an age range of 5 to 14 years old who were enrolled in a primary school in KKM. The school participated in the MDA program to support the elimination of STH infections. However, five years post-MDA program (2018–2022), no follow-up program had been established to evaluate the administration's effectiveness. Subsequently, through this study, investigations for IPI in general were initiated by the School of Medicine and Health Sciences (SMHS), Universitas Katolik Indonesia Atma Jaya (UKIAJ), Jakarta, Indonesia, which gained full support from the North Jakarta health authority. The explanation of the study purpose was socialized to the primary school and KKM Public Health Center (PHC) officers, followed by the distribution of informed consent to the student's parents. A total of a minimal 220 samples were needed for this study, which was based on the calculation using the G*Power 3.1.9.7 software [37]. All active students from this primary school were encouraged to participate in this study voluntarily with consent from their parents or guardians. A total of 338 out of 747 students were given consent to participate in this study, which involved stool examinations, blood tests for hemoglobin levels, and measurements of height and body weight. Out of 338 students, 289 parents or guardians attended seminars on hygiene and sanitation and were interviewed using the modified WASH questionnaire. Consequently, a total of 289 pairs of parents and children who have completed data for both rt-PCR diagnoses and filled questionnaires were further included in the paired analysis.

Specimens' collection

The stool specimens were collected during March 2023. Prior to the distribution of a labeled, sealed stool container and a plastic bag to secure the filled stool container, an explanation and demonstration on how to properly self-collect the stool specimens were performed on a class-per-class basis to ensure that each student could provide a sufficient amount of stool specimens (approximately 10 mL) and of appropriate quality, including the absence of maggots, mixing with water or urine, and contamination with tissue or cotton. The students were instructed to collect their fresh stool specimens (≤24 hours after defecation) once, accompanied by signed informed consent forms from their parents or guardians. The specimens were deposited at the school within the five-day timeframe of sampling. Subsequently, the collected specimens were transported daily to the Parasitology Laboratory at SMHS-UKIAJ for further examination.

Anthropometric measurements (height and weight) of each student who participated in this study were assessed and utilized to calculate *Z*-scores to attain body mass index (BMI)-for-age according to WHO guidelines for ages 5–19 years [38]. BMI values were further categorized into the *Z*-score BMI-for-age classification by the WHO: severe thinness (<-3 SD), thinness (-3 SD<*Z*-score<-2 SD), normal (-2 SD<*Z*-score<+1 SD), overweight (+1 SD<*Z*-score<+2 SD), and obese (>+2 SD) [38].

Hemoglobin (Hb) concentration was measured from finger-prick blood. Quick-Check™ Plus test strips were employed to measure Hb levels according to the manufacturer's recommendations. A single drop of blood from a pricked finger was placed on the test strip, resulting in the Hb level in g/dL. The cut-off values for normal Hb levels were divided for children aged 5–11 and 12–14 years. Children aged 5–11 and 12–14 years were classified as anemic if their Hb levels were below 11.5 g/dL and 12 g/dL, respectively. Hb levels lower than the threshold value were further classified into mild anemia (11–11.4 g/dL for 5–11 years old; 11–11.9 g/dL for 12–14 years old), moderate anemia (8–10.9 g/dL), and severe anemia (<8 g/dL) [39]. The individual-obtained data was collectively reported to the school, Kamal Muara PHC, and North Jakarta Public Health Office (PHO) to enable the local authorities to take further action for students with abnormal BMI and anemia.

Assessment of intestinal parasitic infections (IPIs)

Upon arrival at the Parasitology Laboratory SMHS-UKIAJ, each appropriate stool specimen was used for microscopic examination and to prepare aliquots. The stool specimen aliquots of approximately 1–1.5 g were immediately stored at -80°C for deoxyribonucleic acid (DNA) extraction and rt-PCR detection. Three microscopy procedures, i.e., direct smear, Harada Mori, and Kato-Katz smear, were performed on the collection day.

Microscopic examination

To perform a direct smear, stool was dispensed into 1–2 drops of 1% lugol solution on the object glass, followed by the observation and recording of cysts, eggs, larvae, or other forms of parasites. The Harada Mori procedure was employed to detect the larvae of hookworms and *S. stercoralis*. A freshly obtained stool was spread with a wooden spatula over 4–5 cm in the middle of a tapered end filter paper and subsequently deposited into a plastic bag. Approximately 5 mL of sterile distilled water was inserted through the filter paper, with the tapered end of the paper touching the bottom of the plastic bag, and incubated at 30°C for ten days, followed by microscopic examination [40,41]. The Kato-Katz was performed using a template correlating with 41.7 mg of stool. A small amount of stool was filtered using a filter cloth and transferred into the template. The stool was further covered in malachite-green cellophane, smeared, and examined under the microscope. Observed worm eggs were counted and multiplied by 24 to obtain the eggs per gram (EPG) number [41]. The infection status data were summarized and reported to the school, Kamal Muara PHC, and North Jakarta PHO, allowing the authorities to take further action for disease management.

DNA extraction

The DNA extraction from stool specimens was performed using the spin column-based method (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany) as previously described [25]. In brief, 100 mg of stool was suspended in 200 µL of phosphate-buffered saline containing 2%

polyvinylpolypyrolidone. The suspension was heated for 10 minutes at 100°C and treated with ATL buffer containing proteinase K for 2 hours at 55°C. The internal control for the DNA extraction was phocine herpesvirus 1 (PhHV-1) spiked into AL buffer. At the end of the extraction, DNA from each specimen was eluted with 200 µL of AE buffer.

Real-time polymerase chain reaction (rt-PCR)

Modification of two previously designed multiplex rt-PCR detection panels was utilized to detect and quantify specific DNA [25], targeting six species of intestinal protozoa. Panel 1 targets *D. fragilis, G. intestinalis,* and *Cryptosporidium* spp.; Panel 2 targets *Blastocystis* spp., *E. histolytica,* and *E. dispar*. The list of primers-probes sets used in panel DGC and BHD were summarized in the **Underlying Data**. Each designated panel was dispensed with 5 µL of the DNA template and 20 µL of the master mixture reagents. A positive control for each targeted species and a negative control (H_2O) were included in each run. A short centrifugation was performed, followed by inserting the plate into the pre-configured PCR according to the previous study [25]. The amplification and detection processes were conducted using the CFX96 Touch Real-Time PCR Detection System [Bio-Rad]. The rt-PCR method also offered a semi-quantitative measure using cycle threshold (Ct) value to detect parasite DNA. The Ct value analysis was performed using Bio-Rad CFX Maestro Ver. 4.1.2433.1219. The DNA load distribution was categorized into low ($35 \leq Ct < 50$), moderate ($30 \leq Ct < 35$), and high ($Ct < 30$) [25].

Water access, sanitation, and hygiene (WASH) questionnaire

Upon sampling completion (stool, anthropometric, and blood), the research team invited the parents of participating students to attend an interview session with trained enumerators regarding the modified WASH questionnaire. To minimize any potential biases, the data was obtained by interviewing the parents to align their perceptions with the questionnaire content. The questionnaire used in this study was adapted and translated into Bahasa from the WHO's WASH standardized questionnaire in English, with modifications based on the official Environmental Health Risk Assessment (ERHA) by the MoH and a previously published study, that considered Indonesia's WASH conditions [19,42,43]. The modified questionnaire addressed additional aspects related to water accessibility, sanitation conditions, and hygiene practices for each participant, which serve as risk indicators for IPIs. Since the questionnaire combined questions from all three standardized, well-established questionnaires used in other studies or official surveys, it did not undergo validity and reliability tests. The questionnaire contained four sections: socio-demographic data (age, residence, relationship between respondents and students, education and occupation of parents); water access (water source for drinking, cooking, washing dishes, and bathing, time required to access water, difficulty in accessing water, treatment of drinking and cooking water, and storage of treated water); sanitation (latrine ownership, sanitation facilities, and stool disposal); and hygiene (hand washing habit before meal, hand washing with soap, and hand washing habit after defecation). The detailed modified WASH questionnaire used in this study is provided in the **Underlying Data**.

Data management and analysis

Descriptive analysis was conducted to present demographic characteristics, the prevalence of IPPIs based on microscopy and rt-PCR, WASH profiles, nutritional status, and hemoglobin levels. Prior to bivariate analysis, several variables were re-coded into groups of different variables. Parents' education was re-categorized into "middle school or less" and "high school or above" to differentiate the educational level of parents. Indonesia has mandated nine years of education since 1994; starting in 2015, this requirement has been extended to twelve years. Therefore, parents who undergo education for less than 9 years are considered to have a low educational level [44]. The parent's current occupation was categorized into two groups: no employment or self-employed (fisherman, entrepreneur, freelancer) and employed (private employee and public employee). Water sources and stool disposal were re-categorized into "high-risk" and "low-risk" based on the WHO survey criteria and other relevant studies [19,45]. High-risk refers to a group of WASH components that do not meet WHO standards and are therefore assumed to have a higher chance of infection. Conversely, low-risk is a group of WASH components that are in accordance with WHO standards, thus assumed to provide better protection from infections.

Water sources that were classified as high-risk included “tap water from a peddler,” “public tap water,” “unprotected dug well,” and any combination between low-risk and high-risk. Meanwhile, “packaged water,” “refilled water,” “household tap water,” “boreholes water,” and “protected dug wells” were categorized as low-risk water sources. Low-risk methods for stool disposal included "septic tank" and "sewer pipe/system," while high-risk methods included "ground hole/pit," "drainage," and "open area (river/lake/pond/etc.).” Difficulty in accessing water, water treatment, and handwashing practice were recorded into two categories, such as “never” and “once in a while”; “unprocessed” and “boiled”; and “sometimes/never” and “always”, respectively. The nutritional status classification was categorized according to the WHO classification (described above in the “Specimens’ collection” section); it was grouped into two categories: “underweight” and “normal/overweight/obese”. The anemia status was also re-categorized into “normal” and “anemia”. In the bivariate analysis, only the infection status of the students, either positive or negative, based on rt-PCR results was used to analyze the association between IPPIs and other factors. The Chi-square test was employed to investigate whether the IPIs were affected by any of the collected demographic characteristics and WASH, as well as to evaluate the association between parasite infections and nutritional status. A *p*-value of less than 0.05 was considered statistically significant, and an odds ratio (OR) with a 95% confidence interval (CI) was calculated to determine the associations of the analyzed variables. The collected data were inputted into the Microsoft Excel database and subsequently imported into the Statistical Package for the Social Sciences (SPSS) Ver. 27 (IBM Corp., Armonk, New York, USA). Graphics were visualized using GraphPad Prism 10.

Results

Demographic characteristics, nutritional status, and anemia status

This study involved a total of 338 students who provided parental informed consent and submitted stool specimens, followed by 289 parents/guardians who attended the interview session on the WASH questionnaire. The sex distribution among students was 49.7% (168/338) boys and 50.3% (170/338) girls. The student age group was divided into two categories: 5–10 years old, which includes children mostly in grades 1–3 of primary school, and ages 11–14 years old, which includes children in grades 4–6 of primary school or those who entered school later than the intended age or did not advance to the next grade level. This age classification was established based on previous research that demonstrated the susceptibility of children aged 5–10 years to malnutrition as a result of helminth infections [46]. The majority (61.6%) of participants comprised children aged 11–14 years old, averaging 10.67±1.7 years old. The vast majority of children (89%) resided in KKM, which was one of the slum areas in the North Jakarta regional administration.

All the data provided by the parents/guardians during the WASH questionnaire interview were screened, checked, and recorded in the database. Missing values or unknown data were excluded from the analysis; thus, the subject’s total may vary for each variable. A vast majority of parents, either mothers (61.9%) or fathers (52.2%) attained an educational level of middle school or less. Most of the father’s occupation was recorded as employed (60.4%; 162/268). As for mothers’ occupations, it was mainly listed as unemployed/self-employed (87.4%; 250/286) (**Table 1**).

Based on the analysis of students' nutritional and anemia statuses, the finding shows that a total of 178 (61.6%) students had a normal BMI. Additionally, 30 (10.4%) students were classified as overweight, and 31 (10.7%) students were obese. However, 50 (17.3%) of students were identified as underweight. Regarding anemia status, a significant proportion of students exhibited normal hemoglobin levels (94.8%; 274/289); nonetheless, five students (1.7%) were classified as having mild anemia, and 10 students (3.5%) had moderate anemia (**Table 1**).

Table 1. Factors associated with intestinal protozoa parasitic infections (IPPIs)

Parameters (n=289)		Parasitic infection, n (%)		Total, n (%)	Odds ratio (95%CI)	*p*-value
		Negative	Positive			
Age of student	11–14 years old	66 (47.1)	45 (30.2)	111 (38.4)	1	
	5–10 years old	74 (52.9)	104 (69.8)	178 (61.6)	2.06 (1.27–3.33)	0.002
Sex of student	Girl	68 (48.6)	75 (50.3)	143 (49.5)	1	
	Boy	72 (51.4)	74 (49.7)	146 (50.5)	0.93 (0.58–1.47)	0.428
Mother's education (n=286)*	High school or above	48 (34.5)	61 (41.5)	109 (38.1)	1	0.138
	Middle school or less	91 (65.5)	86 (58.5)	177 (61.9)	0.74 (0.46–1.20)	
Father's education (n=274)*	High school or above	67 (51.1)	64 (44.8)	131 (47.8)	1	0.174
	Middle school or less	64 (48.9)	79 (55.2)	143 (52.2)	1.29 (0.80–2.07)	
Mother's occupation (n=286)*	No employment/self-employed	124 (89.9)	126 (85.1)	250 (87.4)	1	0.153
	Employed	14 (10.1)	22 (14.9)	36 (12.6)	1.54 (0.75–3.16)	
Father's occupation (n=268)	No employment/self-employed	53 (41.1)	53 (38.1)	106 (39.6)	1	0.356
	Employed	76 (58.9)	86 (61.9)	162 (60.4)	1.13 (0.69–1.84)	
Anemia status	Anemia	6 (4.3)	9 (6.0)	15 (5.2)	1	0.344
	Normal	134 (95.7)	140 (94.0)	274 (94.8)	0.69 (0.24–2.01)	
Nutritional status	Underweight	24 (17.1)	26 (17.4)	50 (17.3)	1	0.535
	Normal and above	116 (82.9)	123 (82.6)	239 (82.7)	0.97 (0.53–1.80)	
Drinking and cooking water source	High-risk water	28 (20)	28 (18.8)	56 (19.4)	1	0.456
	Low-risk water	112 (80)	121 (81.2)	233 (80.6)	1.08 (0.60–1.93)	
Washing dishes and showering water source	High-risk water	22 (15.7)	21 (14.1)	43 (14.9)	1	0.743
	Low-risk water	118 (84.3)	128 (85.9)	246 (85.1)	1.13 (0.59–2.17)	
Time duration to access water	≥5 minutes	6 (4.3)	9 (6.0)	15 (5.2)	1	0.344
	<5 minutes	134 (95.7)	140 (94.0)	274 (94.8)	0.69 (0.24–2.01)	
Difficulty in accessing water	Once in a while	2 (1.4)	4 (2.7)	6 (2.1)	1	0.372
	Never	138 (98.6)	145 (97.3)	283 (97.9)	0.52 (0.09–2.91)	
Treatment of drinking water (n=272)*	Boiled	71 (53.8)	70 (50)	141 (51.8)	1	0.307
	Unprocessed	61 (46.2)	70 (50)	131 (48.2)	0.85 (0.53–1.38)	
Store boiled water (n=141)	Yes	69 (97.2)	69 (98.6)	138 (97.9)	1	
	No	2 (2.8)	1 (1.4)	3 (2.1)	2.00 (0.17–22.57)	0.505
Treatment of cooking water (n=287)*	Boiled	105 (75.5)	127(85.8)	232 (80.8)	1	0.035
	Unprocessed	34 (24.5)	21 (14.2)	55 (19.2)	1.95 (1.07–3.57)	
Store boiled water (n=228)*	Yes	87 (84.5)	112 (89.6)	199 (87.3)	1	
	No	16 (15.5)	13 (10.4)	29 (12.7)	1.58 (0.72–3.46)	0.318
Latrine ownership	Have latrine	134 (95.7)	140 (94.0)	274 (94.8)	1	
	No latrine	6 (4.3)	9 (6.0)	15 (5.2)	0.69 (0.24–2.01)	0.344
Sanitation facilities	Gooseneck toilet	137 (97.9)	145 (97.3)	282 (97.6)	1	1.000
	Plunge toilet/empty/gone toilet	3 (2.1)	4 (2.7)	7 (2.4)	1.26 (0.27–5.73)	
Stool disposal (n=284)*	Low-risk	113 (81.3)	116 (80)	229 (80.6)	1	0.450
	High-risk	26 (18.7)	29 (20)	55 (19.4)	0.92 [0.51–1.65)	
Hand wash before eating	Always	125 (89.3)	139 (93.3)	264 (91.3)	1	0.159
	Sometimes/never	15 (10.7)	10 (6.7)	25 (8.7)	1.66 [0.72–3.84)	
Hand wash with soap	Always	107 (76.4)	121 (81.2)	228 (78.9)	1	0.197
	Sometimes/never	33 (23.6)	28 (18.8)	61 (21.1)	1.33 [0.75–2.34)	
Hand wash after defecation	Always	131 (93.6)	145 (97.3)	276 (95.5)	1	0.105
	Sometimes/never	9 (6.4)	4 (2.7)	13 (4.5)	2.49 [0.74–8.27)	

* Unknown information or missing values were excluded from the analysis

Prevalence of the intestinal parasitic infection

A total of 338 students' stool specimens were examined. However, 4, 8, and 18 specimens were unavailable to be examined with direct smear, Harada Mori, and Kato-Katz, respectively, due to insufficient stool or the presence of contaminants in the stool. Three microscopic examinations revealed no STH infection, but some IPPI infections were detected using the direct smear method. Therefore, the molecular-based examination proceeded with only two intestinal protozoan panels using rt-PCR. Regarding rt-PCR detection, the inhibition was encountered in 23 specimens. Therefore, it resulted in 315 specimens with available PCR data.

Detection of IPPI using both the direct smear and rt-PCR methods is presented in **Table 2**. Examination of 334 fecal specimens by direct smear revealed that 61 participants (18.3%) were diagnosed as positive for IPPIs, with *Blastocystis* spp. being the most prevalent (12.0%; 40/334). The other identified species were *Cryptosporidium* spp., *E. coli*, *G. intestinalis*, and *E. histolytica/dispar*. Further examination utilizing an advanced molecular-based method, rt-PCR, was carried out on 315 specimens to diagnose the intestinal protozoan infection. The overall prevalence of IPPI among 315 students was 52.4% (165/315). In accordance with the microscopic findings by direct smear, the predominant infection identified by rt-PCR examination was caused by *Blastocystis* spp. (48.6%; 153/315). Furthermore, IPPIs detected were *G. intestinalis*, *D. fragilis*, *E. dispar*, and *Cryptosporidium* spp. in 21, 13, 9, and 5 children, respectively. The *E. histolytica* (0.3%) infection was found to be the lowest.

Table 2. Comparison between the detection of intestinal protozoa parasites using direct smear and real-time polymerase chain reaction (rt-PCR)

Species	Number of positive cases, n (%)	
	Direct smear (n=334)	rt-PCR (n=315)
Blastocystis spp.	40 (12)	153 (48.6)
Entamoeba histolytica	2 (0.6)**	1 (0.3)
Entamoeba dispar		9 (2.9)
Giardia intestinalis	2 (0.6)	21 (6.7)
Dientamoeba fragilis	0 (0)	13 (4.1)
Cryptosporidium spp.	17 (5.1)	5 (1.6)
Entamoeba coli	3 (0.9)	N/A
Any IPPI infection*	61 (18.3)	165 (52.4)

IPPI: Intestinal Protozoan Parasitic Infection; N/A: Not applicable
*Single infection or coinfection of any species of IPPI
**_E. histolytica/dispar_ is often difficult to differentiate during the dormant cyst stage under a microscope [47]

Further assessment was conducted on the paired data from microscopy and rt-PCR (n=315) to compare the results of both methods. The discrepancies between positive results in the microscopy and rt-PCR were observed in 28 specimens. A total of 13 out of 28 specimens, rt-PCR revealed different intestinal protozoa species than the one that was determined by microscopy, while 15 specimens were negative by rt-PCR despite a positive direct smear detection. Out of the 13 specimens that tested positive for *Cryptosporidium* spp. using direct smear: nine specimens tested positive for DNA of *Blastocystis* spp. and one tested positive for *D. fragilis*, while three specimens yielded negative results. In the case of 12 specimens that were *Blastocystis* spp. positive by direct smear: one specimen turned out to be *Cryptosporidium* spp. positive by rt-PCR, whereas no targeted DNA of intestinal protozoa was amplified in the remaining 11 specimens. Additionally, two specimens were reported to be positive for *E. histolytica/dispar* by microscopy, but both of them were detected to be *Blastocystis* spp. positive following rt-PCR detection. Lastly, a specimen was detected to be infected with both *Blastocystis* spp. and *Cryptosporidium* spp. by direct smear, despite being rt-PCR negative for any DNA target species. Altogether, this suggests the significance of molecular-based methods in confirming the infection status.

The rt-PCR was determined to be superior due to its increased sensitivity and ability to provide semi-quantitative Ct values that reflect the intensity of infection. The detection of *Blastocystis* spp. resulted in a high DNA load in 97 out of 153 (63.4%) positive specimens. Moderate DNA load and low DNA load were detected in 22 (14.4%) and 34 (22.2%) positive specimens, respectively. Out of 21 positive specimens of *G. intestinalis*, six (28.6%) demonstrated high DNA load, four (19.0%) had a moderate DNA load, and 11 (52.4%) specimens had a low DNA load. Based on 13 positive specimens for *D. fragilis,* four (30.8%) exhibited a high DNA load, five

(38.4%) displayed moderate concentrations, and four (30.8%) had low concentrations. Out of nine positive specimens of *E. dispar*, seven (77.8%;) specimens had a low DNA load, two (22.2%) specimens had a high DNA load, and none with a moderate DNA load were detected. Two specimens were discovered with a high DNA load of *Cryptosporidium* spp., one with a moderate DNA load, and two with a low DNA load. Even though *E. histolytica* was only positive in one specimen, and the DNA load of the parasite was classified as high. DNA load distribution of the detected intestinal protozoa was summarized in **Figure 1**.

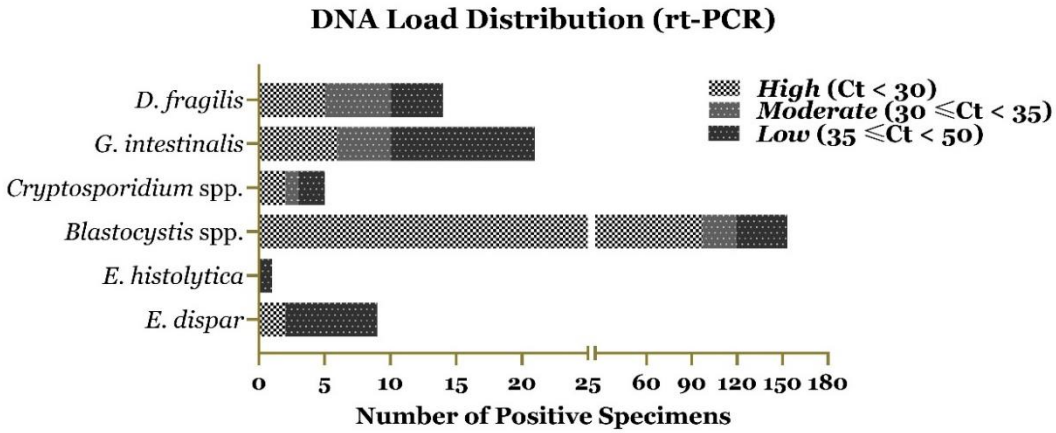

Figure 1. DNA load distribution of six intestinal protozoan parasites using real-time polymerase chain reaction (rt-PCR). Ct: cycle threshold.

Respondent's characteristic, nutritional status, and anemia status association with intestinal protozoa parasitic infection (IPPI)

Age demonstrated a significant association with IPPI (**Table 1**). The risk of infection was found to be higher among children aged 5–10 years old than those aged 11–14 years old (OR=2.06; 95%CI=1.27–3.33; p=0.002). Other demographic factors, such as sex, parents' educational level and occupation, and students' nutritional status, were not significantly associated with IPPIs. Although the results for some parameters were not significant, the analysis revealed the tendency of students with normal/overweight/obese BMIs (OR= 0.97; 95%CI= 0.53–1.80; p=0.535) and normal hemoglobin level (OR= 0.69; 95%CI= 0.24–2.01; p=0.344) to be less likely to get IPPIs.

Water access, sanitation, and hygiene (WASH) profile association with IPPIs

The modified WASH questionnaires assessed water access, sanitation, and hygiene conditions in 289 households. Over a quarter of participants (26.3%) used refilled water as their source of drinking water and household tap water for cooking. The majority of households (63%) also used tap water as a showering or washing dishwater source (**Underlying Data**). Most of the households used low-risk water sources for drinking and cooking (80.6%) and for washing the dishes and showering (85.1%) (**Table 1**).

The vast majority of the respondents were able to access water in less than 5 minutes (94.8%) and never had difficulty accessing water (97.9%). Participants predominantly treated their drinking water (51.8%) and cooking water (80.8%) by boiling and subsequently storing it. Several households with packaged and refilled drinking water did not boil their water source before drinking, assuming that the packaged water is generally securely sealed and safe for direct consumption. The participant's habit of treating drinking water had no significant association with IPPIs, whereas treated cooking water had a significant association (OR=1.95; 95%CI=1.07–3.57; p=0.035). The complete WASH profile of participants is displayed in **Table 1**. Further analysis was conducted by selecting cases to analyze the treated cooking water sources. It was revealed that most participants with improved cooking water sources who did not treat their water had a higher chance of infection (OR=2.25; 95%CI=1.12–4.50; p=0.026) (**Table 3**).

Almost every household had a latrine (94.8%) and defecated using a gooseneck-type closet (97.6%). The ownership of latrines did not present any significant association with IPPIs (OR=0.69; 95%CI=0.24–2.01; p=0.344). Only seven respondents used the plunge/empty/gone toilet, and most of them (57.1%; 4/7) were found to be infected with IPPIs; however, there was no significant association (OR=1.26; 95%CI=0.27–5.73; p=1.000). Despite the fact that the most

prevalent methods of stool disposal were categorized as low-risk (septic tank and sewer pipe), there was no significant association between the method and IPPIs (OR=0.92; 95%CI=0.51–1.65; *p*=0.450), as presented in **Table 1**.

Table 3. The correlation between water sources, water treatments, and the occurrence of IPPIs in households

Variables	Parasitic infection, n (%)		Odds ratio (95%CI)	*p*-value
	Negative	Positive		
Treatment of drinking water (High-risk water source) (n=54)*				
Boiled	19 (73.1)	15 (53.6)	1	
Unprocessed	7 (26.9)	13 (46.4)	0.42 (0.13–1.33)	0.167
Treatment of drinking water (Low-risk water source) (n=218)*				
Boiled	52 (49.1)	55 (49.1)	1	
Unprocessed	54 (50.9)	57 (50.9)	1.00 (0.58–1.70)	1.000
Treatment of cooking water (High-risk water source) (n=56)*				
Boiled	21 (75.0)	22 (78.6)	1	
Unprocessed	7 (25.0)	6 (21.4)	1.22 (0.35–4.23)	1.000
Treatment of cooking water (Low-risk water source) (n=231)*				
Boiled	84 (75.7)	105 (87.5)	1	
Unprocessed	27 (24.3)	15 (12.5)	2.25 (1.12–4.50)	0.026

*Unknown information or missing values were excluded from the analysis

According to parent information in the hygiene section (**Table 1**), the majority of students maintain good hygiene practices and frequently wash their hands. Most of the children always wash their hands before meals (91.3%), with soap (78.9%), and after defecation (95.5%). However, no significant association was found between students practicing hand hygiene practices and those who do not always conduct such practice, i.e., only occasionally wash their hands before meals (OR=1.66; 95%CI=0.72–3.84; *p*=0.159), using soap (OR=1.33; 95%CI=0.75–2.34; *p*=0.197), and after defecation (OR=2.49; 95%CI=0.74–8.27; *p*=0.105) with IPPIs in this population.

Discussion

To our knowledge, this is the first study to investigate the relationship between WASH, and IPIs (including IPPIs) in Indonesia using an advanced diagnostics technique, rt-PCR. The study focused on the link between WASH and IPIs in an urban slum setting, Kamal Muara, North Jakarta District, with a particular emphasis on children. The microscopic examination to detect helminth infection was conducted using the Harada Mori and Kato-Katz methods. The Harada Mori Filter Paper Culture (HMFPC) method is advantageous because it can easily identify third-stage larvae of hookworm and *S. stercoralis*, making it highly sensitive [48]. On the other hand, the Kato-Katz thick smear is a standard method recommended by WHO to detect STHs, including *A. lumbricoides, T. trichiura*, and hookworm, and is often used for mapping and monitoring national control programs [49]. Despite employing both described STH detection methods in addition to the direct smear method, no STH infection was identified. This result is similar to a study carried out in another peri-urban setting [13].

A study in an area of North Jakarta reported that the prevalence of helminth infection was 10.76% [7], contrary to the current study's findings (0%). The disparity in the prevalence of STH infections between a previous study and this current investigation is more likely due to minor changes in the Harada Mori method employed in the two studies; the present study adhered to the WHO-established standard [41], whereas the other used different amounts of stool specimens for smearing on the filter paper. However, the most plausible explanation for the discrepancy may be that the previous study was conducted in 2019, shortly after the initiation of the MDA program, whereas the present study was conducted at a primary school that has been participating in the MDA program for deworming for the past five years. Therefore, consistent treatment could have eliminated the helminth infection. Previously in 2016, prior to the program, a study with a similar population setting performed in the South Jakarta slum area revealed a low prevalence of

helminth infection (1.2%; 2/157) [50]. In accordance with the present investigation, the MDA program's implementation might have contributed to the elimination of helminths in recent years. In addition to the national MDA program to control helminth infection, several factors of hygiene practices, including effective handwashing and access to improved defecation facilities, may have attributed to the STH elimination in the current study.

In contrast to STH, protozoan infections were observed in this study population. The direct smear examination revealed an IPPI prevalence of 18.3%, with *Blastocystis* spp. infection contributing as the most common case (12.0%) and *E. histolytica/dispar* as the least common infection (0.6%). Protozoan infections were previously observed in another slum area of North Jakarta among school-age children, revealing a prevalence of 2.8%, 0.5%, and 0.5% for *G. intestinalis*, *E. histolytica/dispar*, and *E. coli,* respectively, which were investigated using direct smear method [51]. Another study conducted in a primary school located in South Jakarta, also affected by flooding, reported a higher prevalence of protozoan infection (35%), with *Blastocystis* spp. as the most dominant species (28%) [50]. The disparity between the two studies may be due to the floods in KKM, North Jakarta being primarily caused by the intrusion of saline seawater, especially during high tide, whereas the floods in South Jakarta were mainly caused by the blockage of river water due to accumulation of waste. *Blastocystis*'s lower prevalence in North Jakarta might be due to its cyst's lack of resistance to seawater or chlorine. However, there is a potential for the cyst to develop resistance to chlorine exposure, which raises uncertainty about the effectiveness of chlorine in killing the organism [52]. Regardless of the circumstances, the cyst form of *Blastocystis* spp. is the most common due to its resistance to environmental factors [53]. Given the debatable pathogenicity of *Blastocystis* spp., the high prevalence of *Blastocystis* spp., in the current investigation was predicted. Based on a review of the current status of *Blastocystis* spp. infection in humans, the prevalence can range from 5% and can reach up to 80% in developing countries [54]. It has been determined that *Blastocystis* spp. may be the causative agent of functional gastrointestinal disorder (FGID) [55]. *Blastocystis* spp. infection rates are influenced by many factors, including the host's immune status, underlying medical disorder, and living environment. The immune system and age are inextricably linked; children possess a less developed immune system than adults. Consequently, children may be more susceptible to *Blastocystis* spp. infection than adults [56].

The utilization of rt-PCR in this study resulted in a nearly three-fold higher prevalence of protozoan infections compared to the direct smear method. A previous study in Cuba also indicated an almost two-fold higher percentage of IPPIs detected by the rt-PCR method compared to the microscopic method [57]. This outcome is apparent because molecular-based methods have a greater sensitivity and specificity compared to conventional approaches, such as microscopy [58]. The rt-PCR provides a semi-quantitative result that corresponds to the intensity of the targeted pathogens as determined by the Ct value, reflecting the parasite-specific DNA load. Lower Ct values indicated a greater abundance of the targeted parasites [23]. Moreover, the specificity of rt-PCR enables the differentiation of *E. histolytica* and *E. dispar*, two morphologically similar protozoa species found in this current study. It is crucial to distinguish both species, as *E. histolytica* is the pathogenic agent that causes intestinal amoebiasis [57]; thus, accurately diagnosing *E. histolytica* is key for effectively treating the infected patient [59]. The current result also revealed a 63.4% *Blastocystis* spp. positive cases had a high DNA load. Within those positive cases, it remained necessary to understand the subtype distribution of *Blastocystis* spp., as it might further explain the controversy of the pathogenic versus non-pathogenic specific subtypes of this particular protozoan [60]. The differentiation of these subtypes might offer essential information due to their possible association with conditions such as irritable bowel syndrome with diarrhea (IBS-D) [61]. *Blastocystis* spp., subtype 3 (ST3), is frequently detected in patients with persistent gastrointestinal disorders. Distinct clinical situations and abundance and diversity of gut microbiota are closely associated with different subtypes of *Blastocystis* spp. Therefore, in order to gain a comprehensive understanding of the *Blastocystis* subtype's function and significance in the gut microbiome of humans, additional research is necessary to investigate their distribution and impact [56].

The current study identified 5.1% and 1.6% of *Cryptosporidium* spp. by microscopy and rt-PCR, respectively, even though rt-PCR revealed the preponderance of infected participants. This

contradictory outcome suggests the potential for false positive results when analyzing *Cryptosporidium* spp. using direct smear microscopy. The direct smear method's lack of a specific Lugol's dye could potentially obstruct the inspection of the true *Cryptosporidium* spp. oocyst, masking it from other unwanted components [62]. Alternatively, a modified Ziehl-Neelsen (mZN) acid-fast stain is the recommended method for detecting *Cryptosporidium* spp. under a microscope, as adding an acid component is useful for identifying coccidian species oocysts [63,64]. Conversely, the high sensitivity and specificity of rt-PCR in this study revealed the presence of true positive *Cryptosporidium* spp. infections (1.6%) among students. Despite the potential for false negatives [65,66], this study consistently identified a greater number of positive infections of other protozoan species using rt-PCR than the microscopic method, thereby demonstrating the potential of rt-PCR to detect IPPIs.

This present study analyzed the association of children's age with parasitic infection, showing that children between the ages of 5 and 10 have a higher risk of developing IPPIs. A significant association was observed between the protozoan infection and age group. Children below 10 years old may have less knowledge about hygiene compared to children above 10 years old [67], which might contribute to the higher risk of IPPIs. Previous research has shown an association between age and IPPIs, with children in lower age groups being more susceptible to the infection [68]. It was also examined that children under 12 years old tend to be more active in outdoor activities involving soil, thus making them more susceptible to infection through the fecal-oral route [69]. The previous and current results implied that age is a significant risk factor for IPPIs. In addition to children's age, children's habits may also differ among the sexes. However, the analysis revealed there was no significant association between sex and IPPIs (**Table 1**), although there was a slight disparity in prevalence between girls (52.4%) and boys (50.7%). Parasites complete their life cycle in humans regardless of sex, thus exposing all hosts to an equal risk of infection. Nevertheless, various factors might increase the likelihood of infection in individuals, i.e. individuals' activities in indoor or outdoor areas, lifestyle choices, and failure to follow recommended health practices, such as wearing shoes outside or refraining from nail clipping [70].

Socioeconomic status (SES), such as the educational level and current occupation of parents, is also one of the contributing factors to IPI. Parental education is important for fostering awareness of the importance of hygiene; however, it is challenging to translate knowledge and attitudes into behavior in the absence of adequate facilities. This study did not find any association between parents' education level and IPPIs, nor between parental occupation and IPPIs. The result is comparable to a study conducted in peri-urban areas, which found no association between parental education and occupation with IPPIs [71]. On the contrary, an earlier cross-sectional study found a significant association between parents' education and occupation with their children's infection status; the infections were higher among children whose parents, either the mother or father, have a lower education level and certain jobs (housewife for mother and worker for father) [72]. The nonsignificant outcome of the present study might suggest that all social classes were presumably exposed to parasites at the same rate due to homogeneous environmental conditions [73]. In future research, it will be important to observe additional potential sources of infection, such as drinking water at the point of use, ready-to-eat foods, and courtyard soil from children's outdoor play areas. According to a prior investigation, *E. coli* was frequently found in the home environment (drinking water, ready-to-eat foods, and courtyard soil) and it was strongly associated with IPIs in children [74]. Furthermore, independent of the socioeconomic status of their parents, all KKM residents have access to government-funded social healthcare services in hospitals, irrespective of their parent's socioeconomic status [75].

Along with demographic factors and SES, there are also health issues that may arise from the infections by protozoa and helminths, such as wasting and growth retardation that affect children's nutritional status, alongside anemia [14,15,76]. Hookworm infections can cause anemia and protein deficiency because their attachment to the human intestine could result in blood loss among infected individuals [77]. The current study did not find any helminth infections among the children. Moreover, intestinal protozoa do not cause manifestations as severe as helminth infections. Hence, the study reveals that most of the students possess a normal

nutritional status and hemoglobin levels, as indicated in **Table 1**. However, this study identified a number of students who were underweight and had anemia conditions. Their circumstances might be influenced strongly by other factors than WASH, such as poverty, nutrient deficiency, insufficient dietary consumption, genetic factors or diseases, and other undetected infectious agents [78-81]. Further investigation is necessary due to the interrelatedness of these factors, which could potentially impact urban transition.

Intestinal parasites are transmitted through the fecal-oral route, typically through contaminated water or food, which is the most common source [51]. In particular, the consumption of contaminated water is frequently associated with protozoan infection. Most KKM residents were found to have an improved water source (80.6%) with the majority of the respondents using household tap water. Despite the categorization of water sources into low-risk and high-risk infections, there were no differences in both categories as neither showed a significant association with IPPIs. This is in contrast to a study conducted in Adadle, Ethiopia, which stated the significant association between high-risk drinking water sources—rainwater and river—and *G. intestinalis* infection [82]. The differences observed in the KKM and Adadle areas may be attributed to variations in water sources, as most KKM households use improved water sources.

There was no significant association between treated drinking water sources and IPPIs. This finding is similar to other studies [42,83,84]. This outcome is in opposition to the findings summarized by Fieberkorn *et al.* [85]. It may suggest that drinking water treatment is inefficient in this community [84]. Other than drinking water, the respondents also seemed to boil and store their cooking water in a closed container. The habit of not boiling cooking water had a significant association with IPPIs (OR=1.95; 95%CI=1.07–3.57; p=0.035). A sub-analysis was also performed to observe the conditions in two different subpopulations: respondents with low-risk water sources and those with high-risk water sources. Whether they use a low-risk or high-risk water source, it was discovered that the treatment of drinking water did not significantly associate with IPPIs. On the contrary, there was a strong association between IPPIs and unprocessed low-risk cooking water sources (OR=2.25; 95%CI=1.12–4.50; p=0.026) (**Table 3**). The inconsistent results regarding the impact of boiling treatment of cooking water and infection rates among participants may be ascribed to other factors. This study did not examine the likelihood of hazardous pathogen exposure to treated cooking water during water storage, nor did it explore the appropriate implementation of the boiling technique in each household. As suggested by the Centers for Disease Control and Prevention (CDC), water should be boiled for one minute, set until cold, and stored in a closed container. It is highly possible that the parasites in the water will be eliminated if the boiling technique is carried out according to the established guidelines. Boiling water before consumption is one of the many ways to prevent parasite, viral, and bacterial infections; thus, boiled water might have a lower infection risk [86]. Other variables in this study, such as respondents' difficulty in accessing water and the duration to obtain water, were stated to have no significant association with IPPIs. The nonsignificant results might be due to the homogenous conditions regarding both parameters, as almost all households easily obtained water for their daily activities. Nonetheless, this research observed a trend of higher positive parasite infections among the students who experienced difficulty in collecting water (66.7%; 4/6) and longer duration of fetching the water (100%; 4/4).

This study demonstrated an absence of association between protozoa infections and latrine ownership. This might be due to the similar nature of the study respondents, in which most of the households already have improved sanitation facilities, with only a small proportion having inadequate latrine facilities. Similar findings from a previous study in an urban slum in Brazil showed that the presence of a private latrine within the household did not correlate with intestinal parasitic infection [87]. Despite the absence of a connection to the IPPIs, insufficient sanitation facilities may facilitate the transmission of the disease to other children by contaminating the environment. Consequently, it is advisable to prioritize environmental development and other preventive measures that focus on the improvement of WASH, particularly in urban slums [7].

Children in KKM mostly implemented good hygiene practices, according to the parents' information. These proper practices could contribute to the absence of an association between hand hygiene practices and IPPIs in this study. By practicing good personal hygiene during

crucial moments (before meals, after bathroom usage, after diaper changes, and before and after tending to ill individuals), people are able to protect themselves and others from water-borne intestinal protozoan parasites [32]. Another study indicated an association between the lack of handwashing habits during these crucial activities with protozoan infections (p<0.05; OR=1.3–6.9) [88]. One of the ways to elevate good hygiene habits among children is through parental involvement, as they are the foremost educators of their children. Based on the previous investigation, children exhibit the same level of handwashing behavior and thoroughness of technique as their parents, especially when they spend longer time together [89].

The various implementations of WASH are not only closely related to individual characteristics but could also be influenced by their living environment, such as urban slums. Massive urbanization from rural to urban settings is responsible for the rise of slums in developing countries [90]. The transmission and management of parasitic infections are substantially influenced by the setting in urban slums for several reasons. Initially, the high population density and closely adjoined housing conditions facilitate the transmission of various parasites through increased person-to-person contact [91]. Furthermore, inadequate infrastructure exacerbates disparities among residents, particularly in accessing essential WASH components. The lack of proper infrastructure further hinders effective management and prevention efforts for parasitic infections [91].

Although the present study found that an adequate WASH profile is generally displayed by the respondents, IPPIs still remain a health concern in this study area. Multiple factors, including insufficient understanding, treatment, and prevention of IPPIs, as well as inconsistent adherence to maintaining proper WASH, especially hygiene, by children, might explain the current infections found in this study. Hence, in order to prevent the unprecedented transmission of IPPIs among schoolchildren, it is imperative that multilevel stakeholders, including the government and parents, actively contribute to raising health awareness regarding IPPIs and associated risk factors, which include ensuring sufficient WASH facilities and practices [92]. Moreover, host immunity plays a role in determining the incidence of parasitic infections, as certain protozoa, such as *Blastocystis* spp. and *Cryptosporidium* spp., mainly infect immunocompromised hosts. Moreover, the presence of IPPIs may reduce the efficacy of the rotavirus vaccine and subsequently produce detrimental effects on human hosts, particularly children [93].

It is evident that adequate WASH is a critical component in the fight against the transmission and increasing cases of communicable diseases [94], despite the nonsignificant association between the majority of WASH aspects and IPPIs. Consequently, the third and sixth SDGs are contingent upon the effective administration of WASH [95]. Furthermore, WASH could provide additional protection to children who have received the rotavirus vaccine. According to a study conducted in Peru, the incidence of diarrheal clinic visits is lower among post-vaccinated children who have access to clean water and sanitation facilities compared to the pre-vaccine era [96].

Several limitations were present in this investigation. Firstly, there was a lack of direct observation of the households' WASH profile, which included microscopically examining the water sources and directly observing the condition of sanitation facilities and the hygiene practices of the children. The observation is critical for providing valuable insight into local WASH-related behaviors and supporting the data from the structured questionnaire [97]. This will provide a more comprehensive understanding of the WASH condition in urban areas. Second, the study took place in a school that had implemented a deworming program, resulting in a zero prevalence of helminth infection. This limited the association between the parasites and the clinical manifestations, as protozoa are most frequently asymptomatic. Nevertheless, the strength of our study lies in the integration of three separate surveys, each covering a wide range of WASH aspects. Additionally, our IPPI findings were strengthened by the utilization of rt-PCR. This method will produce a more accurate measure of IPPIs than the microscopic examination.

Conclusion

Our study demonstrates the superior efficacy of the rt-PCR method in detecting IPPIs, as molecular detection yielded a prevalence nearly three times higher than that detected by microscopy. Interestingly, the high prevalence of IPPIs observed in this study contrasts with the

collected data on Water Access, Sanitation, and Hygiene (WASH) practices. The population in this study demonstrated a generally improved WASH profile, despite residing in a slum area. However, several households still utilized high-risk water sources for consumption, lacked private toilet facilities – necessitating the use of shared toilets or, in some cases, open defecation – and employed unsafe stool disposal practices. The results suggest that self-reported improvements in WASH practices do not necessarily guarantee the elimination of protozoan infections. The presence of at least one of the IPPIs in over half of the participants underscores the persistent health concerns associated with urban slum areas, likely attributable to multiple contributing factors. Among these, younger age was identified as a significant factor influencing IPPIs. To address health issues by these infections, there is still a need to implement consistently good WASH practices, especially for younger children. Untreated cooking water was shown to be significantly associated with IPPIs; therefore, treating water with the proper procedures prior to consumption is important to control IPPIs. Moreover, it is imperative to acknowledge the potential adverse impacts of IPPIs, including their potential to compromise the efficacy of vaccines and thus undermine children's immunity. Given the presence of certain opportunistic infections, further investigation is warranted to explore their clinical implications in this context. Lastly, to provide a comprehensive understanding of IPIs in urban settings, additional studies are needed not only in other slum areas of Jakarta but also in other urbanized regions. Such studies would help comprehensively grasp the association between IPI and urban slum settings, thereby informing the development and implementation of effective interventions to reduce infection rates. Consequently, comprehensive monitoring and control of IPIs in urban slums are essential for advancing public health outcomes.

Ethics approval

This study was approved by the Ethical Committee of the School of Medicine and Health Sciences (SMHS), Universitas Katolik Indonesia Atma Jaya (UKIAJ), under reference #26/03/KEP-FKIKUAJ/2023. The research was obtained as recommended by the Jakarta Provincial Government under document # 1/AF.1b/31.72/2/TM.23.04/e2023.

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Competing interests

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Underlying data

The supplementary data and table can be accessed at http://doi.org/10.6084/m9.figshare.27208200.

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