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Prevalence of intestinal protozoan infections and association with hygiene knowledge among primary schoolchildren in Salahutu and Leihitu districts, Central Maluku regency, Indonesia

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ABSTRACT. The objectives of this study were to estimate the prevalence of intestinal protozoan infections and to determine the association with hygiene knowledge among primary schoolchildren in Central Maluku regency, Indonesia. A cross-sectional study was conducted among fourth- and fifth-grade primary schoolchildren during May to June 2015. Stool specimens were examined using direct and modified Ziehl–Neelsen staining methods. Hygiene knowledge was obtained from a pre-tested questionnaire. Data were analyzed using SPSS® version 22.0 statistic software. Of the 170 students surveyed, 69 (40.6%) were infected with one (32.9%) or more (7.7%) intestinal protozoans. The age ranged from 8 to less than 15 years old. The most frequent protozoan detected was *Cryptosporidium* sp. (24.7%), followed by *Entamoeba coli* (13.5%), *Giardia lamblia* (5.9%), *Entamoeba histolytica/Entamoeba dispar* (3.5%), *Chilomastix mesnili* (0.6%), and *Iodamoeba bütschlii* (0.6%). The most frequently detected multi-infection was *Cryptosporidium* sp. with *E. coli* (4.1%). Only 10 of 170 students had lack of knowledge regarding hygiene, nevertheless this is a significant risk factor for intestinal protozoan infections ($P < 0.05$). Education on personal and environmental hygiene should be improved and implemented to prevent and control intestinal protozoan infections in this population.

INTRODUCTION

Intestinal protozoan infections occur as a result of infection with single or multiple intestinal parasites, such as *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* (Sedjini *et al.*, 2011; Wegayehu *et al.*, 2013; Haftu *et al.*, 2014). These infections are caused by ingested food or water that is contaminated with intestinal protozoan cysts or by direct fecal-oral transmission. Infections may be asymptomatic or may present with symptoms such as acute diarrhea, chronic diarrhea, dysentery, colitis, liver abscess, and malabsorption (Hill & Nash, 2011; Lima *et al.*, 2011; Peterson *et al.*, 2011). Intestinal protozoan infections can occur at any age but mostly occur in school-age children as a result of indiscriminate defecation or consumption of food or water that is contaminated with intestinal protozoan cysts (Wani *et al.*, 2007; Wegayehu *et al.*, 2013; Haftu *et al.*, 2014).

Intestinal protozoans infect about 3.5 million people worldwide and can result in

morbidity and mortality, especially in developing countries (Vahedi *et al.*, 2012). Intestinal protozoan infection is one of the major diseases in Southeast Asian countries, including Indonesia, for which there are very little prevalence data available (Hotez *et al.*, 2015). The prevalence of intestinal protozoan infection in Central Maluku regency is currently unknown, which is combined with known poor personal and sanitation hygiene (Dinas Kesehatan Provinsi Maluku, 2013).

Inadequate personal hygiene is the primary risk factor for intestinal protozoan infections (Vahedi *et al.*, 2012; Gelaw *et al.*, 2013). A study among aborigine communities in Pahang, Malaysia, showed that poor environmental management, poor personal hygiene, and a lack of health education were associated with a high prevalence of intestinal protozoan infections. Age and sex, however, were not significantly associated with the risk of intestinal protozoan infections. The fact that younger people have a lower level of immunity than those who are older is presumed to lead to a higher rate of intestinal protozoan infections in the young (Azian *et al.*, 2007).

Low personal hygiene in primary schoolchildren, indicated by a lack of hygiene knowledge, leads to a higher risk of intestinal protozoan infections. The objectives of the present study were to determine the prevalence of intestinal protozoan infections among primary schoolchildren in Central Maluku regency, Indonesia and to determine the association of hygiene knowledge with the prevalence of infection.

MATERIALS AND METHODS

Study design and study population

A cross-sectional survey was conducted at two primary schools in Salahutu district and one primary school in Leihitu district, Central Maluku regency, Indonesia. These two districts are directly adjacent to Ambon on Ambon Island. Study subjects and stool specimens were obtained from May until June 2015. Stool specimens were examined at Tulehu General Hospital laboratory, Maluku, and Laboratory of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

This survey was a part of a preliminary study to be undertaken by researchers at the Center for Tropical Medicine, Faculty of Medicine, Universitas Gadjah Mada.

Study subjects and data collection

Minimum sample size was calculated using an estimated proportion of the population formula $n=(Z_{a/2})^2/4d^2$, with an expected prevalence of the true prevalence ±10% (d=0.1) with a 95% confidence level ($Z_{a/2}$=1.96). From this formula, a minimum sample size of 97 samples was obtained (Lemeshow *et al.*, 1997). Study subjects were selected using purposive sampling. Verbal and written research information and a request for consent were provided to students and their parents. Inclusion criteria for the study were that children were fourth- or fifth-grade primary school students within the study area at the time of sampling. Children were excluded if they had or were receiving treatment for intestinal parasites, such as metronidazole, within 2 weeks prior to the date of interview. Exclusion of these children was important to avoid misinterpretation during stool specimen examination.

Age, sex, and hygiene knowledge were obtained by interviewing students using a questionnaire. This questionnaire was tested for validity and reliability prior to fieldwork. Age was calculated based on the provided date of birth and the date of interview, then categorized into two groups: <10 years and ≥10 years. Sex was recorded as male or female. Hygiene knowledge was assessed from 13 multiple-choice questions in the Indonesian language. Questions were asked about: a clean water definition; types of drinking water; things to do before having a meal; knowledge of diseases associated with unclean water; things to do before eating fresh vegetables; things to do before eating fresh fruit; toilet/latrine definitions; clean latrine condition; things to do after defecation, flushing stools after defecation; washing hands after defecation; bathing frequency per day. Each correct answer was scored either 1 for the correct answer or 0 for an incorrect answer. As the previous study for hygiene

knowledge among primary school children in Medan city, North Sumatera province (Syahputri, 2011), total score was catagorized using the median of the total score. In this study, the median was 6.5 from 13 questions. A total score of ≥7 was categorized as adequate hygiene knowledge and a total score <7 as a lack of hygiene knowledge. Before conducting interviews, all interviewers were briefed on how to administer the questionnaire, the choice of answers, and other possible answers they might be given. If any difficulties occurred during interviews, interviewers contacted the principal investigator for clarification.

Stool specimen collection and laboratory examination

To ensure maximum co-operation from study subjects, we informed the principal and teachers of each school about the study and the flow of collecting data from interview and stool specimen collection. Together with each school principal and teachers, we then explained to all children and parents about stool collection. A number of parents needed to assist their children during defecation. We provided parents with a package for stool collection and instructions for the use of the collecting equipment and its functions: an A5-sized sheet of oil paper to use as the base during defecation; a prelabeled 60-mL screw-cap stool container with a spoon-like cap to scoop stool sample; a zipped plastic bag to wrap the container; personal protective equipment (a pair of hand gloves and surgical mask) and instructions for use, instructions for how to collect the stool samples and to avoid contamination with sand or other materials, details of the amount of stool specimen to collect must be around half to three-quarters of the container (about 40 mL), how to confirm that the container was sealed correctly, how to dispose of all equipment except the container with plastic after collection, and the appropriate time to return the container. Packages for stool specimen collection with written instructions were distributed to each student after the interview. Students were asked to return the containers with fresh stool specimens to school within 3 days of the interview. The stool sample was collected once for each subject.

All specimens were brought to the laboratory of Tulehu General Hospital for identification, then preserved in 10% formalin. Each sample was examined in Tulehu Laboratory Hospital. During shipping by plane, all preserved specimens were transported safely by cargo service with double-sealed plastics for each container and covered with bubble wrap. Some of the samples were also randomly cross-checked to the parasitology laboratory, Faculty of Medicine, Universitas Gadjah Mada for quality control.

Intestinal protozoa were identified from stool specimens by light microscope examination using direct staining methods with Lugol to allows identification of protozoan based on its morphology. The examination was performed twice each by different laboratory technicians. Further confirmation was provided by examination using modified Ziehl–Neelsen staining by another laboratory technician. Stool specimens were stained and examined according to World Health Organization standard procedures (World Health Organization, 2012). Intestinal protozoan infection was defined as the presence of any cyst or trophozoite of an intestinal protozoan observed from staining procedures. A single infection was defined as the presence of only one species of intestinal protozoan, and multi-infections were defined from the presence of one or more species of intestinal protozoans. Species identified were confirmed directly by a senior laboratory technician from the Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada. As morphology of *E. histolytica* is indistinguishable from *E. dispar* using direct examination, laboratory recording was *E. histolytica/E. dispar*. Morphology cyst of *E. histolytica/E. dispar* is distinguishable from *E. coli* by nuclear morphology and its size. *Entamoeba coli* cyst are greater than 15 mm and often have five to eight nuclei, whereas *E. histolytica/ E. dispar* cyst have less than five nuclei and usually 10-15 mm in diameter (Peterson *et*

al., 2011). Modified Ziehl-Neelsen staining provides identification for acid-fast protozoans, including oocyst from many species of *Cryptosporidium*; laboratory recording was therefore as *Cryptosporidium* sp. (Lima *et al*., 2011).

Data analysis

All data analysis was performed using SPSS® version 22.0 (SPSS Inc., 2002). The prevalence and types of intestinal protozoan infections were obtained from stool examination results using descriptive statistics. Questionnaire data without available stool specimens were excluded. Associations between age, sex, hygiene knowledge and intestinal protozoan infections were analyzed using the chi-square test. Results with an associated $P<0.05$ were considered statistically significant. Relative risk between age, sex, hygiene knowledge and intestinal protozoan infections was estimated as a prevalence ratio with 95% confidence intervals (Sastroasmoro *et al*., 2014).

Ethical considerations

This study, including the collection of human stool samples, was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada-General Hospital Dr. Sardjito No. KE/FK/567/EC/2015. This study collaborated with local health authority by referring subjects with positive pathogenic protozoan results for further examination and treatment.

RESULTS

There was a total of 197 students at three primary schools; 18 students did not participate in an interview, three students had received treatment for parasites in the previous 2 weeks, and six students did not submit stool specimens. Of the 170 study participants, 84 students were from SD Negeri 11 from Tulehu, 32 students from SD Negeri 02 Tengah-Tengah in Salahutu district, and 54 students from SD Negeri Oli in Leihitu district (Figure 1). The age of participants ranged from 8 to <15 years. From the 170 participants, there were 34 students aged <10 years, 80 male students. Only 10 students had a lack of hygiene knowledge.

Table 1 shows the prevalence of pathogen and non-pathogen intestinal protozoans identified at the different schools. Generally, the most common pathogenic intestinal protozoan identified was *Cryptosporidium* sp. (24.7%), especially in SD Negeri 11 Tulehu, SD Negeri 02 Tengah-Tengah, and SD Negeri Oli (respectively 19.0%, 50.0%, and 18.5%, respectively). Generally, the most common non-pathogenic intestinal protozoan identified was *E. coli* (13.5%), especially in SD Negeri 11 Tulehu, SD Negeri 02 Tengah-Tengah, and SD Negeri Oli (12.1%, 15.6%, and 13.0%, respectively).

Table 2 shows the types and occurrences of intestinal protozoan infections in total and from each school. A total of 69 students (40.6%) were infected with intestinal protozoans. Infections were present in the form of single infections (32.9%) and as multi-infections (7.6%). The most common single infection was *Cryptosporidium* sp. infection (19.4%). The most common multi-infection was *Cryptosporidium* sp. with *E. coli* (4.1%).

Figure 2 shows the most commonly incorrect answer for each question regarding hygiene knowledge. The question answered most incorrectly was question number 8 regarding healthy latrine condition.

Table 3 shows the association between age, sex, hygiene knowledge, and intestinal protozoan infections. Intestinal protozoan infections were more prevalent in children aged <10 years (47.1%) than children aged ≥10 years (39.0%), but this association was not statistically significant ($P=0.390$). Intestinal protozoan infections were slightly more prevalent in female (41.1%) than male (40.0%) individuals, but this association was also not statistically significant ($P=0.883$). Intestinal protozoan infections were significantly more prevalent in children with a lack of hygiene knowledge (80.0%) than in children with adequate hygiene knowledge (38.1%), corresponding to a prevalence ratio of 2.098 (95% confidence interval 1.453–3.030, $P=0.016$).

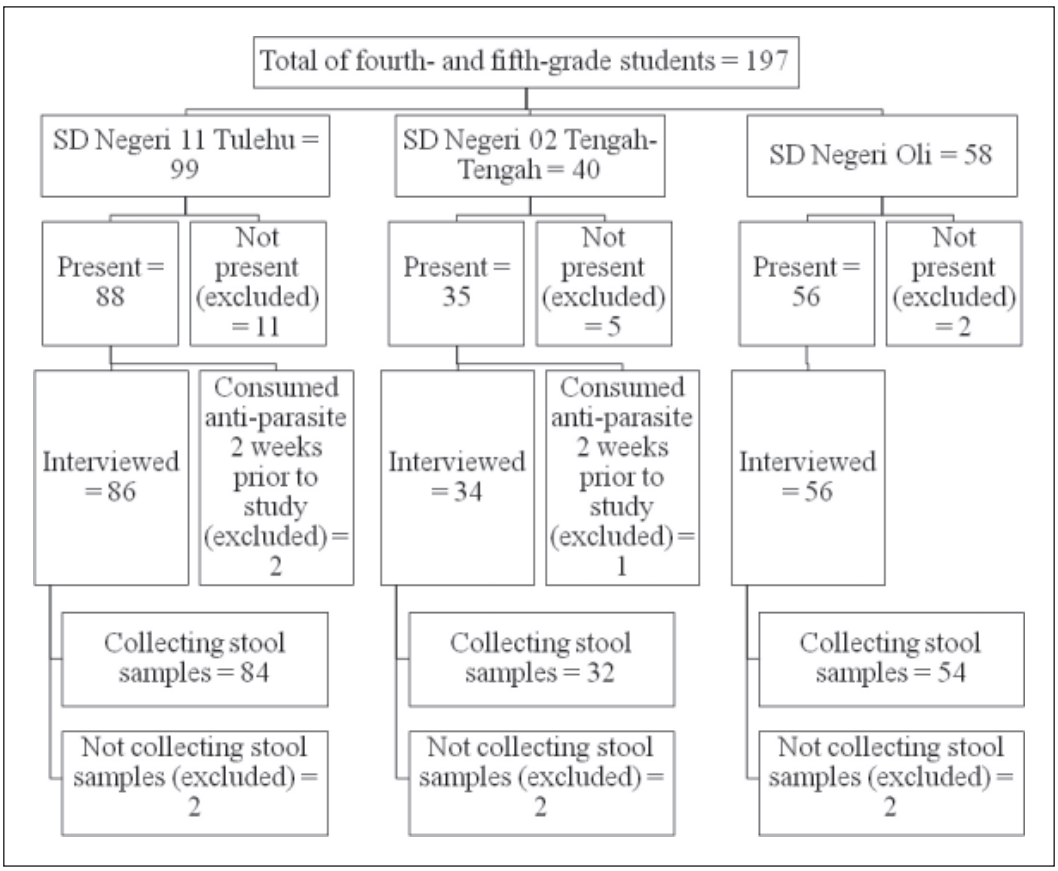

Figure 1. Subjects participation scheme.

Table 1. Prevalence of intestinal protozoan infections

Intestinal protozoans	Total (N=170)		SD Negeri 11 Tulehu (N=84)		SD Negeri 02 Tengah-Tengah (N=32)		SD Negeri Oli (N=54)	
	n	%	n	%	n	%	n	%
Pathogen:								
E. histolytica / E.dispar dispar	6	3.5	5	6.0	1	3.1	0	0.0
G. lamblia	10	5.9	3	3.6	1	3.1	6	11.1
Cryptosporidium sp.	42	24.7	16	19.0	16	50.0	10	18.5
Non pathogen:								
E. coli	23	13.5	11	13.1	5	15.6	7	13.0
C. mesnili	1	0.6	1	1.2	0	0.0	0	0.0
I. bütschlii	1	0.6	1	1.2	0	0.0	0	0.0

N = number of examined, n = number of infected

Table 2. Types and occurences of intestinal protozoan infections

Types of intestinal protozoan infections	Total (N=170)		SD Negeri 11 Tulehu (N=84)		SD Negeri 02 Tengah-Tengah (N=32)		SD Negeri Oli (N=54)	
	n	%	n	%	n	%	n	%
Single infection:	56	32.9	22	26.2	17	53.1	17	31.45
E. histolytica /E. dispar	3	1.8	3	3.6	0	0	0	0
G. lamblia	6	3.5	1	1.2	1	3.1	4	7.4
Cryptosporidium sp.	33	19.4	12	14.3	13	40.6	8	14.8
E. coli	13	7.6	5	6	3	9.4	5	9.25
I. bütschlii	1	0.6	1	1.2	0	0	0	0
Multi-infections:	13	7.7	7	8.3	3	9.4	3	5.55
E. histolytica /E. dispar and *Cryptosporidium* sp.	1	0.6	0	0	1	3.1	0	0
E. histolytica /E. dispar and *E. coli*	1	0.6	1	1.2	0	0	0	0
G. lamblia and *Cryptosporidium* sp.	1	0.6	0	0.0	0	0	1	1.85
G. lamblia and *E. coli*	1	0.6	0	0.0	0	0	1	1.85
G. lamblia and *C. mesnili*	1	0.6	1	1.2	0	0	0	0
Cryptosporidium sp. and *E. coli*	7	4.1	4	4.8	2	6.3	1	1.85
E. histolytica /E. dispar, *G. lamblia* and *E. coli*	1	0.6	1	1.2	0	0	0	0
Total	69	40.6	29	34.5	20	62.5	20	37

N = number of examined, n = number of infected

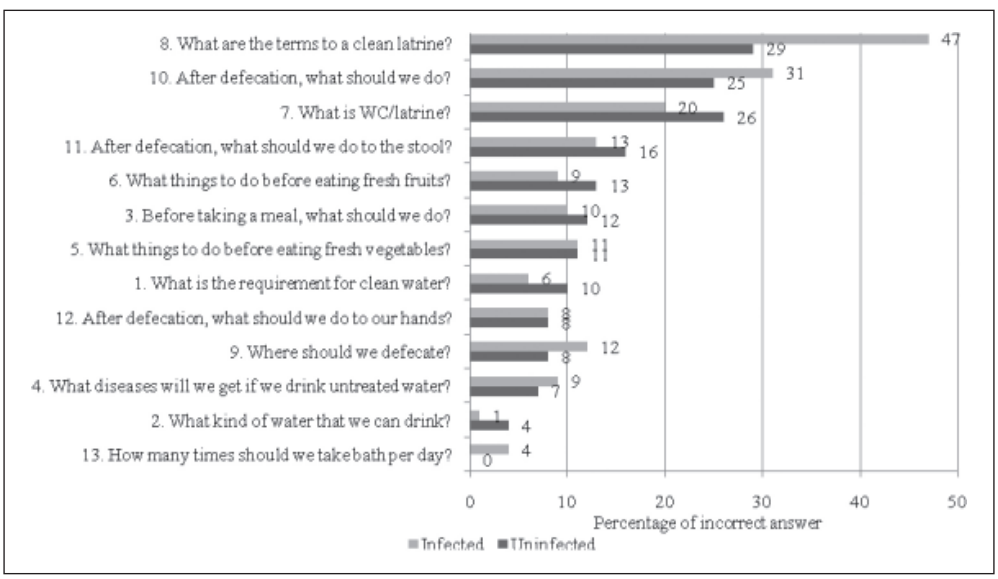

Figure 2. Frequency distribution of incorrect answers by infections status for 13 questions about hygiene knowledge related to intestinal protozoan infection

Table 3. Association between age, sex, hygiene knowledge, and prevalence of intestinal protozoan infections

Subject characteristics	Intestinal protozoan infections						
	Yes		No		*P*	PR	95% Confidence Interval
	n	%	n	%			
Age:							
< 10 years old	16	47.1	18	52.9	0.390	1.208	0.789 – 1.827
≥ 10 years old	53	39.0	83	61.0			
Gender:							
Male	32	40.0	48	60	0.883	0.973	0.675 – 1.401
Female	37	41.1	53	58.9			
Hygiene knowledge:							
Inadequate/lack	8	80.0	2	20.0	**0.016**[1]	2.098	1.453 – 3.030
Adequate	61	38.1	99	61.9			

[1] Using the Fisher exact test because there were 1 or more cells with expected values less than 5, $P<0.05$ was statistically significant, PR = prevalence ratio.

DISCUSSION

The prevalence of intestinal protozoan infections among primary schoolchildren in Central Maluku regency, Indonesia was high; up to 40.6% in the form of single infections (32.9%) and multi-infections (7.7%). The prevalence in other areas of Indonesia varied in rural and urban areas. A study among primary schoolchildren carried out by Irawan *et al*. (2002) in Kepulauan Seribu observed a high prevalence of *G. lamblia* (30%) and low prevalence of *E. histolytica* and *E. coli* (5%). A study carried out by Darnely *et al*. (2011) among children in Bekasi observed different prevalences for *G. lamblia* (7%) and *E. coli* (3%). The study by Mandela *et al*. (2013), observed a prevalence of *Cryptosporidium* sp. in children with diarrhea in Pekanbaru of 10%.

The present study found a high prevalence of intestinal protozoa, with *Cryptosporidium* sp. (24.7%), followed by *E. coli* (13.5%), *G. lamblia* (5.9%), and *E. histolytica/E. dispar* (3.5%). These results indicate a high risk of intestinal protozoan infections among the population, especially in schoolchildren, as found in other studies (Irawan *et al*., 2002; Darnely *et al*., 2011). Owing to the high prevalence of intestinal protozoan infections, health workers, communities, and governments in Central Maluku regency must increase awareness of risk factors for intestinal protozoan infections.

A lack of hygiene knowledge suggests poor hygiene behavior, as shown by earlier studies where poor hygiene behavior has been shown to increase the risk of intestinal protozoan infections. Indiscriminate defecation has been shown to increase risk 2.4–38.2 times, poor drinking water 11.5 times, poor hand washing 5.7–6.45 times, and taking a bath less than twice per week 1.820 times (Quihui *et al*., 2006; Wani *et al*., 2007; Alyousefi *et al*., 2011; Ngui *et al*., 2011; Gelaw *et al*., 2012; Haftu *et al*., 2014).

Hygiene knowledge among primary schoolchildren in this study generally was adequate. There were only 10 of 170 students who had a lack of hygiene knowledge. Nevertheless, lack of hygiene knowledge was still a significant risk factor for intestinal protozoan infection. Hygiene knowledge in this study was assessed using 13 questions related to the knowledge of clean water, drinking water, hand washing, hygiene of food and drinks, defecation, and frequency of taking a bath. A question regarding the components of a healthy latrine was the most incorrect answer, indicating indiscriminate

defecation behavior such as defecating directly into a river, on a beach, in a garden, or without use of a septic tank.

Hygiene knowledge is required to avoid the risk of intestinal protozoan infection, especially about healthy latrine conditions. Education on personal and environmental hygiene should be improved and implemented to prevent and control intestinal protozoan infections.

All children aged 8 to <15 years in this area had similar daily activities. Intestinal protozoan infections can infect all ages, but mostly school-age children (Azian *et al.*, 2007; Gelaw *et al.*, 2012). Age and sex were not significant factors for intestinal protozoan infections (Azian *et al.*, 2007; Ngrenngarmlert *et al.*, 2007; Wani *et al.*, 2007; Wegayehu *et al.*, 2013).

The limitations of this study were the limitations in species identification using microscopic examination, examination with preserved stool samples that could not identify trophozoite movement for a treatment decision, restriction of study subjects to fourth- and fifth-grade primary schoolchildren, and the inability to directly observe attitudes and actions reflecting hygiene behavior.

CONCLUSIONS

The prevalence of intestinal protozoan infections in Central Maluku regency was high with a lack of hygiene knowledge as a significant risk factor. It is important to improve and to implement personal and environmental hygiene, especially related to healthy latrines, to prevent and control intestinal protozoan infections.

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