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### Conflicts of Interest

No conflicts of interest.

### Author Contribution

Conceptualization: SW. Data curation: VPP.  
Formal analysis: SW, SPP. Funding acquisition: SS. Investigation: SW, SS. Methodology: SW, SS. Project administration: VPP. Resources: SW, VPP, SS. Software: SW, VPP. Supervision:

# The Prevalence of Intestinal Protozoan Infections among Children in Southwest Sumba Based on the Type of Water Sources

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## ABSTRACT

**Background:** Intestinal protozoan infection is a public health problem in Indonesia, particularly in regions lacking clean water sources such as Perokonda and Perobatang villages, Southwest Sumba Regency, East Nusa Tenggara Province. The residents of Perokonda and Perobatang villages use wells and unprotected open springs respectively. Therefore, this study aims to determine the prevalence of intestinal protozoan infections in these two villages.

**Materials and Methods:** This was a cross-sectional study, carried out in Perokonda and Perobatang from August 2016 to August 2017. A total of 291 children aged 6 months to 18 years were used as subjects. Furthermore, fecal examinations were carried out by preparing smears, which were stained with lugol solution and examined under the microscopes by four experienced technicians. The data were processed using SPSS version 20 and analyzed by chi-square test.

**Results:** Among the 291 samples, the prevalence of intestinal protozoan infections was 20%, with 23.4% in Perokonda and 17.2% in Perobatang. The predominant species found were *Blastocystis hominis* (34.5%), *Giardia lamblia* (19.0%), and *Entamoeba coli* (15.5%). Moreover, approximately 69.0% of single infections were found while 31.0% were mixed and associated with 2 to 3 protozoan species. There were no significant differences between the prevalence of intestinal protozoan infections and the children in Perokonda and Perobatang villages (confidence interval 0.83 - 2.62,  $P = 0.19$ ).

**Conclusion:** The type of water sources has no relationship with the species of intestinal protozoan infecting children in Perokonda and Perobatang.

**Keywords:** Intestinal protozoan; Children 6 months to 18 years old; Water sources; Southwest Sumba

## INTRODUCTION

Parasitic intestinal infections caused by worms and protozoa affect the health of Indonesians both in urban and rural areas [1, 2]. Meanwhile, the risk factors leading to these infections include consumption of uncooked meat fresh vegetables, contaminated hands, limited access to clean water, open field defecation [3, 4], habitual or regular contact with dirt, and the



SS. Validation: SW, SS. Visualization: SW, VPP, SS. Writing - original draft: SW, VPP. Writing - review & editing: SS.

number of family members living in a single house [5]. Furthermore, intestinal protozoan infects individuals across all age range but are more prevalent in children due to the inability to maintain personal hygiene. These infections are transmitted from animals, food, or beverages contaminated with a protozoan cyst or through human to human fecal-oral contact [3, 4].

The most common causative organisms of intestinal protozoan infections are *Giardia lamblia*, *Entamoeba histolytica* *dispar*, and *Cryptosporidium* spp. [3-5]. Sarkasi et al. [6] stated that the prevalence of pathogenic and non-pathogenic protozoan in southwest Iran reached 37.5% where the predominant species was *Endolimax nana* at 21.07%. Furthermore, a study by Sungkar et al. [7] in Kalena Rongo, Southwest Sumba, found *Blastocystis hominis* (34.4%), *G. lamblia* (17.9%), and *E. histolytica* (4.5%).

The Perokonda and Perobatang villages are classified as under-developed regions, where 28.1% of the population are poor [8]. These regions also have fewer sanitation facilities with limited access to clean water. The 2013 Basic Health Research (*Riskesdas*) showed that 97.9% of households have limited access to sanitation facilities, 54.6% do not have proper indoor toilets, hence, making Southwest Sumba the second-highest regency in the East Nusa Tenggara Province. Approximately 39.2% of households still use outdoor floorless makeshift toilets, 22.4% use water from open and unprotected springs, 15% use rainwater collected in reservoirs, while 11.9% use water from the local river for household needs [9]. These water sources are quite vulnerable to contamination from trash or garbage, as well as chemical substances, and diseases including parasites [10].

The residents of Perokonda village use wells while the residents of Perobatang village use unprotected springs as water sources to meet different needs. Meanwhile, the low hygiene level, lack of sanitation facilities, and limited access to clean water might correlate with the types of parasites infecting the population of Southwest Sumba. Therefore, this study aims to determine the prevalence of intestinal protozoan infections among the population of Perokonda and Perobatang villages based on the different water sources used for household needs.

## MATERIALS AND METHODS

### 1. Research location

The Perokonda and Perobatang villages are located in Kodi subdistrict, Southwest Sumba Regency. Perokonda's residents get water from a well (Fig. 1A). A water pump is installed in the well and it draws water through a pipe to water tanks equipped with faucets, located at the edge of the main road. There are 12 water tanks with a 30-meter space between each other. These arrangements enable the residents to get water for daily needs because the tanks were very close. Meanwhile, in Perobatang, there was only one natural spring and it is not protected from the element (Fig. 1B). The residents collect the water for daily needs and wash clothes at the spring.

### 2. Population

This cross-sectional study was carried out in Perokonda and Perobatang villages Kodi subdistrict, Southwest Sumba Regency, East Nusa Tenggara Province, Indonesia. The population in Perokonda and Perobatang consists of 65 and 35 families respectively. Furthermore, the number of children in each household varies widely from 1 to 14 per household with an average of 6 per household. The inclusion criteria include residents between the age of 6 months to 18 years old that live in Perokonda or Perobatang, present during data collection,



**Figure 1.** (A) A well and water tank where Perokonda residents get the water for daily needs. (B) The unprotected water source used in Perobatang.

have parental consent for participating in the study, and returned the pots containing feces. Meanwhile, the exclusion criteria include children with serious illnesses at that time.

### 3. Sample size

The number of minimum samples required for the study was calculated using the single proportion sample size formula to determine the estimated proportion of a population:

$$n = \frac{(z\alpha)^2 PQ}{d^2}$$

Remarks:

- $n$  = the number of required samples
- $\alpha$  = generalization error, set at 5%
- Value  $z\alpha = 5\%$  of  $\alpha$  standard value, is 1.96
- $P$  value = 0.5 based on the prevalence of intestinal protozoan infections in Perokonda and Perobatang villages
- $Q_1$  value =  $1 - 0.5 = 0.5$
- $d$  value = error in predicting the prevalence of intestinal protozoan infections in Perokonda and Perobatang villages, which is still acceptable at 10%

The number of required samples was 96 children from each village, but all children that indicated interest as a subject were included in this study

### 4. Fecal examination for parasites

On the first day, the Perokonda and Perobatang residents were gathered at the respective village head houses for briefings about the upcoming activities. Residents that were willing to participate were interviewed, the biodata were recorded by the researchers, and were given instructions on how to collect fecal samples for the study. Furthermore, the participants were provided with pots to collect the feces sample and were asked to submit the next day. These activities were assisted by the cadres of the local community health centers that acted as interpreters because the residents do not understand Indonesian, but only communicate using the local language. Fecal examinations were carried out by preparing smears, which were stained with lugol solution and examined under microscopes by four experienced technicians. The examination was carried out at the research's location, meanwhile, the researchers had prepared a simple laboratory specifically

for the purpose, while ensuring that safety protocols and good specimen management were fully observed. Intestinal protozoan infection was stated as positive when trophozoites or protozoal cysts were found in the examined fecal matter. Meanwhile, the prevalence of intestinal protozoan infection is the number of residents in Perokonda and Perobatang villages identified as positively infected divided by the total number of samples examined.

### 5. Data analysis and research ethics approval

Ethics approval No. 876/UN2.F1/ETIK/2016 was obtained from the Ethics Committee of the Faculty of Medicine Universitas Indonesia. The data were processed using SPSS version 20.0 (IBM, Armonk, NY, USA) and the Chi-square test was performed to determine the relationship between the prevalence of intestinal protozoan infections and the village of origin.

## RESULTS

A total of 291 children participated in the study and submitted the pots containing feces samples, 128 were from Perokonda while 163 were from Perobatang (**Table 1**). The male subjects were 48.5% while the females were 51.5%. Majority (75.9%) were children of kindergarten and elementary school age (5 to 12 years).

Generally, the prevalence of intestinal protozoan infections among children between 6 months to 18 years old in both villages was 20.0%. Meanwhile, the prevalence value in Perokonda was 23.4%, which was higher than Perobatang at 17.2%. However, there is no relationship between intestinal protozoan infection and the type of water source used for household needs (**Table 2**).

**Table 3** shows that the predominant species found include *B. hominis* (34.5%), *G. lamblia* (19%), and *Escherichia coli* (15.5%). Approximately 69% were single infections while 31% were mixed and associated with 2 to 3 protozoan species.

**Table 1.** Demographic characteristics of the study population

Characteristics	Perokonda (n = 128)	Perobatang (n = 163)
<b>Age</b>		
<5 years old	2 (2%)	47 (29%)
5 - 12 years old	118 (92%)	103 (63%)
>12 years old	8 (6%)	13 (8%)
<b>Gender</b>		
Male	63 (49%)	78 (48%)
Female	65 (51%)	85 (52%)

**Table 2.** The relationship between intestinal protozoan infections and the type of water source for household needs

Village	Intestinal protozoan infections		P-value	95% CI
	Yes	No		
Perokonda	30 (23.4%)	98 (76.6%)	0.19	0.829 – 2.628
Perobatang	28 (17.2%)	135 (82.8%)		

CI, confidence interval.

**Table 3.** Distribution of infections based on species of protozoan

Village	Single Infection			Mixed Infection					
	Bh	Ec	Gl	Bh + Ec	Bh + Gl	Ec + Cc	Gl + Ec	Gl + Cc	Bh + Ec + Eh
Perokonda (n = 30)	13	5	7	1	1	1	1	0	1
Perobatang (n = 28)	7	4	4	7	4	0	0	2	0
Total (%)	20 (34.5)	9 (15.5)	11 (19)	8 (13.8)	5 (8.6)	1 (1.7)	1 (1.7)	2 (3.4)	1 (1.7)

Bh, *Blastocystis hominis*; Ec, *Entamoeba coli*; Gl, *Giardia lamblia*; Cc, *Cyclospora cayetanensis*; Eh, *Entamoeba histolytica/dispar*.

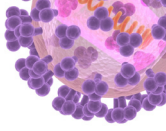
## DISCUSSION

This study was conducted in Perokonda and Perobatang, Southwest Sumba Regency, which has different water sources for household needs. Based on the observation, Perokonda village has a better water source, hence, water availability for the residents was also better. There was a tank filled with water pumped from the well between two or three houses, hence, the residents had access to water for daily needs.

Meanwhile, Perokonda residents live in houses with dirt floors and toilets, nonetheless, the residents still defecate in the open fields. Furthermore, Perobatang village has only unprotected spring, *i.e.* a well in an open field to meet the daily water needs. Meanwhile, water from an open spring is susceptible to contamination, including microorganisms such as protozoa. Perobatang residents have to walk at least 4 km or about 1 to 2 hours on foot to get to the water source. In the spring, the villagers bathe, wash cloths, and return home with one or two jerricans filled with water for daily needs, such as cooking and drinking. The laborious effort to get water led to low personal hygiene, *i.e.* the villagers do not bathe, wash hands regularly nor have proper toilets but defecate in the field. Furthermore, majority of the villagers still live in stilt houses and kept livestock, such as pigs, buffalos, and chickens at the lower part of the house, therefore, there was close contact between humans and animals and this condition creates a fertile ground for parasitic infection.

The prevalence of intestinal protozoan infections is generally high in regions with low economic status and inadequate or poor sanitation condition [11]. In this study, the prevalence was 20%, similar to Yulfi et al. [12] in a study carried out in Samosir island, North Sumatera, Indonesia. However, these figures are slightly higher than the infection rate among children in Sri Lanka [13] but much lower compared to Lebanon [14] and Burkina Faso [15]. These three countries share a common trait in terms of limited access to clean water, as well as low levels of sanitation and personal hygiene. The disparity in prevalence rates is presumably caused by the difference in season or climate and the techniques employed in analyzing the samples. Due to the limited facility at the research location in this study, the researchers had to carry out a direct examination using lugol staining dye, which admittedly limited the detection of more intestinal protozoans types. Moreover, a concentration technique was not performed, given that Sanprasert et al. [16] reported that the difference in sensitivity between a direct examination and the formalin ether concentration technique is not significant (31.2% with direct examination against 33.5% with formalin ether concentration technique). Due to the nature of the analysis, therefore, the fecal examination of the subjects was only carried out once, which affected the result of the parasites examination. Furthermore, examination using the triple-faeces-test method tends to improve the detection of parasitic infections. However, this method and a special dyeing technique are preferably used to examine feces in health facilities [17].

There was no significant difference in the prevalence of intestinal protozoan infections between the two villages. Meanwhile, Yulfi et al. [13] showed that water collected from open sources such as rainwater, lakes or a closed water source, such as an artesian well has no relationship with the occurrence of intestinal protozoan infections. In contrast, Alyousefi et al. [18] reported that the availability of clean water lowers the risk of protozoan infections. The disparities between these studies are due to other risk factors, including low awareness of clean and healthy lifestyle, such as maintaining clean nail and non nail-biting habit [19], not washing hands after defecating and before eating, defecating in an open field and inability to bathe very often [12, 13, 20].



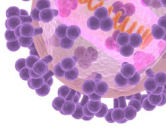
The 2018 Basic Health Research of the Indonesian Ministry of Health [21] stated that only 16.0% of children aged 10 to 14 years in East Nusa Tenggara Province wash hands properly and only 56.7% of the population in Southwest Sumba Regency defecate in toilets. The correct handwashing procedures include the use of soap and running water before preparing food or whenever the hands become dirty after touching money, animals or yard works and gardening, as well as after defecating, washing babies/children, using pesticides/insecticides, or before breastfeeding and before eating [22]. In this study, these risk factors were not considered hence, similar figures of intestinal protozoan infections were obtained from the two villages.

Poor water quality also contributes to the high prevalence of intestinal protozoan infections even though water availability is good. Meanwhile, intestinal protozoan is generally transmitted through the consumption of water contaminated with pathogenic protozoa. Osman et al. [14] reported that the consumption of untreated water triples the risk of protozoan infections compared to water treated with chlorite. In addition, the consumption of uncooked fruits and vegetables after washing with contaminated water tends to also increase the risks of intestinal protozoan infections [23], as well as limited access to clean water and adequate sanitation facilities such as toilets [24]. Similarly, extreme poverty and low economic level are also predictors for protozoan infections occurrence [25, 26].

The prevalence of intestinal protozoan infections in Perokonda and Perobatang was mostly caused by *B. hominis*, followed by *G. lamblia* and then *E. coli*, which is a non-pathogenic protozoan. The number of infections associated with these three types of protozoa was higher in Perokonda, which has a better water supply. These three protozoans are transmitted through the fecal-oral route, therefore, one of the media of transmission is water. Aside from malaria, filariasis, and soil-transmitted helminths, travelers to Indonesia, especially to the Regency of Southwest Sumba needs to be aware of possible protozoan infections caused by *B. hominis* and *G. lamblia* [27]. In this study, the researchers did not examine parasites from water sources used by the residents. Mahardianti et al. [28] reported the presence of *Cryptosporidium* spp. in households water from Ciliwung River in Jakarta. Viable *Cryptosporidium* spp. oocysts were found, suggesting a potential source of protozoan infections from water sources.

*B. hominis* is the predominant protozoan species found in Perokonda and Perobatang. A previous study by Sungkar et al. [7] in Kalena Rongo, Southwest Sumba also indicated that the most common cause of intestinal protozoan infections in the region was *B. hominis*. In addition, a high prevalence of *B. hominis* infections was detected among the primary school children in Central Jakarta [29] and Karang Asem, Bali Province [30]. Several residents in Southwest Sumba do not get adequate nutritional intake, this condition exacerbates the pathogenicity of *B. hominis* infections and might even lead to clinical symptoms such as malnutrition and stunting. Therefore, relevant stakeholders are to take necessary preventive measures towards this species of protozoan [29].

This study also found 18 children infected with *G. lamblia*, either as a single or mixed infection with other protozoan species. *G. lamblia* is a type of protozoan commonly found in water with *Cryptosporidium* spp. [31]; the infection usually lasts from one to two weeks. Moreover, majority of the infections are asymptomatic but also cause clinical manifestations such as diarrhea, steatorrhea, nausea, cramps in the abdominal area, and weight loss. A mild and chronic asymptomatic infection in children leads to malnutrition and development disorder [3, 32]. Asymptomatic giardiasis ranging from 1.8% to 28.9% was discovered by Bachtiar et al. [1] in urban and rural areas in East Java Province. This study also found that the low level



of education among the Southwest Sumba residents contributes to poor disease awareness. Moreover, due to the limited access to health facilities, the villagers only seek treatment when faced with a crippling or life-threatening illness. Currently, only 5% of Southwest Sumba residents have easy access to health facilities [21].

Another parasite found in this study was a non-pathogenic protozoan known as *E. coli*. However, it has been identified as an indicator of environmental pollution due to the poor sanitation and the low hygiene level of the residents [33]. *E. coli* is a common commensal parasite found in the intestinal tract but does not cause clinical symptoms. It is only found in the lumen of the intestinal tract but not in the epithelial cells. Furthermore, *E. coli* infection does not spread to other organs compared to *E. histolytica*. However, the examination of one child in the study showed the presence of *E. histolytica*, therefore, the discovery of *E. coli* in the water needs special consideration since the examination using lugol dyeing technique has its limitation in differentiating Entamoeba species [34].

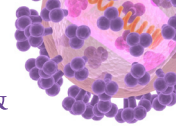
The results also showed *Cyclospora cayetanensis* among children with multi-infections. It is transmitted through water or food contaminated with feces and the infective phase is a sporulated oocyst. These oocysts take several days or weeks under suitable environmental conditions to produce spores, hence, transmission through a direct person-to-person contact has little risk. Furthermore, children, poverty, and low economic conditions are the risk factors for the occurrence of cyclosporiasis [35]. Chavatte et al. [36] identified *C. Cayetanensis* in a patient that came for a regular medical checkup with an immunocompetent status and without any clinical symptom. Similarly, Thima et al. [37] reported 12 positive samples of *C. cayetanensis* from 539 samples of children's feces in Chalerm Phrakiat, Nan Province, Thailand. These samples were collected from children aged 5 to 12 years without a history of chronic fever, vomiting or weight loss.

To reduce the number of intestinal protozoan infections in Southwest Sumba, interventions are to focus on ensuring the availability of clean water, improving sanitation facilities, and periodic education to transform or modify the residents' hygiene behaviors. Lin et al. [38], reported that the implementation of interventions in the form of providing clean water, improving sanitation, and encouraging handwashing at the individual level is more appropriate and cost-effective in rural areas with low per capita income.

In conclusion, the prevalence of intestinal protozoan infections among children aged 6 months to 18 years in Southwest Sumba Regency, East Nusa Tenggara Province is considered to be mild. *B. hominis*, *G. lamblia*, and *E. coli* species were found in single and mixed infections, meanwhile, there was no relationship between the prevalence of intestinal protozoan infections and the water sources used for household needs.

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