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ORIGINAL ARTICLE

Intestinal parasitic infections and associated risk factors: A cross-sectional study in Sunggal District, Deli Serdang

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Abstract

Intestinal protozoan infections have been reported worldwide with highly variable prevalence rates, particularly in tropical and subtropical regions. Despite this, limited research has been conducted on intestinal protozoan infections over the past decade, both in Indonesia and specifically in North Sumatra. Therefore, further investigation into the prevalence and risk factors of intestinal parasitic infections, especially protozoan infections, in areas surrounding Medan is warranted. This cross-sectional study assessed the association between risk factors and intestinal parasitic infections among 136 residents of Sunggal District, Deli Serdang, selected using consecutive sampling. Participants underwent interviews to evaluate risk factors using a checklist and provided fecal samples, as well as livestock samples if applicable. Data were analyzed using the Chi-square test and Fisher's exact test. Results revealed a significant association between age and Protozoa infection ($p = 0.001$), helminth infection ($p = 0.0002$), and overall parasitic infection ($p = 0.0001$). However, no significant associations were found for other variables. Furthermore, sanitation and hygiene levels were not significantly associated with parasitic infections ($p > 0.05$). These findings suggest that the risk factors assessed in this study were not significantly associated with intestinal protozoan infections. Nonetheless, the prevalence of both overall parasitic infections and protozoan infections remains high, with a spectrum ranging from asymptomatic to symptomatic cases. Further research is needed to elucidate the complex interplay of factors contributing to the persistence of these infections in the region.

Keywords: intestinal parasites, intestinal protozoa, feces, sanitation level, hygiene

Introduction

Diarrhea remains a significant global health problem, ranking as the second leading cause of death worldwide and a primary contributor to malnutrition in young children. Over a billion individuals experience acute diarrhea at least once annually. The etiology of diarrhea is multifaceted, with gastrointestinal infections being the most common cause. A variety of pathogens, including viruses, bacteria, and parasites, have been implicated in diarrheal disease.[1,2] In Indonesia, the 2018 Indonesian Health Profile reported an estimated 7,157,483 cases of diarrhea treated in healthcare facilities, with 4,504,524 (62.93%) of these cases receiving medical attention.[3] Intestinal protozoa, such as *Giardia lamblia* (flagellates), *Entamoeba histolytica* (amebae), *Cryptosporidium* (sporozoa), and *Balantidium coli* (ciliates), are a group of parasites known to cause diarrhea. Infections with intestinal protozoa are particularly prevalent in developing countries, where adequate sanitation systems are often lacking.[4]

Giardia lamblia, also known as *Giardia duodenalis* or *Giardia intestinalis,* is a protozoan parasite commonly associated with giardiasis in humans. Approximately 280 million individuals worldwide are estimated to be infected with this parasite.[5,6] Alioes et al.[7] found a prevalence of 22.2% among elementary school students in a Padang school. Entamoeba species are another group of protozoa capable of infecting

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humans, although not all species are pathogenic. Entamoeba histolytica is a well-known human pathogen, causing amebiasis. Primates, including humans, are the primary hosts for E. histolytica, although it can occasionally infect dogs, cats, and pigs.[5,8] The global prevalence of E. histolytica is substantial, with the World Health Organization estimating that around 500 million people are infected, leading to symptomatic disease in 50 million and approximately 100,000 deaths annually.[9] In Indonesia, a previous study reported an amebiasis prevalence of 12.50% in Medan, North Sumatra.[10] Balantidium coli is another common protozoan that causes balantidiasis.[11] Pigs, both domestic and wild, serve as the primary hosts for B. coli, while humans are accidental hosts.[12] The prevalence of B. coli in humans varies widely. In Indonesia, particularly in North Sumatra, a prevalence of 28% has been reported among high-risk populations, such as pig farmers.[13]

Another common, yet generally milder, intestinal protozoan infection in immunocompetent individuals is cryptosporidiosis, caused by the parasite Cryptosporidium. *Cryptosporidium hominis* and *Cryptosporidium parvum* are the most frequent species to infect humans. In individuals with intact immune systems, clinical manifestations can be asymptomatic or present as self-limiting, watery diarrhea. However, severe and chronic manifestations are commonly observed in immunocompromised patients, such as those with AIDS or undergoing immunosuppressive therapy.[14,15] A study of AIDS patients with chronic diarrhea in Jakarta, Indonesia, revealed a prevalence of cryptosporidiosis as high as 36.80%.[16]

A definitive diagnosis of intestinal protozoa can be established by identifying either trophozoites or cysts in microscopic examinations of stool specimens. Unstained preparations are particularly useful for the detection of *Blastocystis hominis* (*B. coli*). Microscopic examination for *B. coli* is typically performed using low power (100X) magnification due to the relatively large size of its cysts and trophozoites.[13] In wet mounts of fresh stool specimens, trophozoites exhibit high motility.[17] Immunoassay and DNA-based methods have also been developed and can differentiate species that are morphologically indistinguishable or difficult to differentiate.[18,19]

Intestinal protozoan infections have been reported in numerous regions worldwide, with a highly variable prevalence, particularly in tropical and subtropical areas.[5] Despite a global distribution, these infections are predominantly classified as neglected diseases. The prevalence of intestinal protozoan infections is significantly influenced by the population of animal hosts, which can serve as reservoirs for these parasites. Primary risk factors for infection include contact with reservoirs, inadequate sanitation, and poor hygiene, facilitating the transmission of protozoan cysts to the human gastrointestinal tract. However, given the asymptomatic nature of many infections, a comprehensive reassessment of other potential risk factors is warranted. Such factors may include demographic characteristics, the presence of symptoms or signs, personal hygiene and sanitation practices, residential conditions, and prior medical history (e.g., underlying diseases, antibiotic use, and contact with potential hosts).

North Sumatra Province is notably the second-largest province in Indonesia in terms of domestic pig population, following East Nusa Tenggara Province, with a recorded 1,228,951 pigs in 2018.[20] Several areas surrounding Medan City have a significant population of residents keeping pigs within residential areas. Pigs are known to serve as hosts for various intestinal protozoa, including *B. coli*, *G. lamblia*, *Entamoeba sp.*[21] Research on intestinal protozoa has been limited over the past decade, resulting in a lack of definitive prevalence data for intestinal protozoal infections in Indonesia, particularly in North Sumatra. Therefore, this study aims to investigate the prevalence of intestinal protozoal infections and identify associated risk factors in areas surrounding Medan City.

Method

This study is a cross-sectional observational study designed to assess the association between diarrhea risk factors and the occurrence of intestinal parasitic infections among residents of Sunggal District, Deli Serdang. All research procedures have been approved by the Medical Research Ethics Committee of the Universitas Sumatera Utara, with approval number 823/KEP/USU/2020. The study population consists of all residents of Sunggal District, Deli Serdang, who possess diarrhea risk factors. A consecutive sampling method was employed to select a sample of 136 individuals from this population. Both inclusion and exclusion criteria were applied to define the sample. Inclusion criteria included individuals residing in environments with poor sanitation who provided written informed consent to participate in the study. Exclusion criteria comprised individuals who had received antibiotic therapy within the past week.

Residents of Sunggal District, Deli Serdang, were provided with an explanation of the proposed study. Those who agreed to participate as study subjects were asked to provide written informed consent. Subsequently, these subjects were interviewed to collect relevant research data. Participants were then instructed on the fecal specimen collection procedure and given a collection kit for both themselves and their livestock.

Fecal specimen collection was conducted using a specialized kit. The process began by ensuring participants wore the provided gloves. A wide-mouthed plastic container was then placed inside the toilet bowl. Participants were instructed to defecate as usual, but before cleaning, they were to remove the plastic container. A small portion of feces was collected from both ends and the middle using a provided plastic spoon and transferred to a sealed container. The sealed container was then given to the on-site staff. After receiving the container, it was labeled with the subject's name, date, and time of collection.

In addition to human fecal specimens, this study also involved laboratory analysis of porcine fecal samples from the study participants' livestock. The collection procedure for porcine feces was similar. Participants were required to wear gloves while collecting the specimen using a wide-mouthed plastic container. Using the provided spoon, a spoonful of feces was collected from both distal ends and the middle, including any liquid or mucus. The container was then sealed and labeled with the livestock owner's name, date, and time of collection.

Fecal specimens from both human and porcine sources were subjected to microscopic fecal examination utilizing two distinct staining methods: Lugol's iodine stain and modified Kinyoun-Gabbett stain. For Lugol's iodine staining, a small sample of feces was collected using an applicator and smeared onto a glass slide previously charged with Lugol's iodine. The slide was then examined under a microscope to identify cysts, trophozoites, and helminth eggs or larvae. The modified Kinyoun-Gabbett staining procedure differed slightly. A small amount of feces was thinly smeared onto a glass slide and allowed to air dry overnight. The smear was fixed with methanol and subsequently immersed in Kinyoun's reagent for 2 minutes, followed by rinsing. A decolorizer, acid alcohol, was applied to remove excess stain, and the slide was rinsed with water. Finally, the smear was immersed in Gabbett's reagent for 5 minutes, rinsed, and allowed to dry. The Kinyoun-Gabbett stained smear was examined under oil immersion (1000x magnification) to identify acid-fast protozoan spores.

Data collection in this study involved the acquisition of primary data through a checklist and microscopic laboratory examination. The checklist was used to identify age, hygiene practices, sanitation conditions, highest level of education, occupation, monthly family income, number of family members residing together, livestock ownership, toilet availability, history of diarrhea within the past week, and history of any diarrhea treatment. Meanwhile, the results of microscopic stool examinations were categorized as protozoal and helminthic infections. Protozoal infections included infections caused by *Entamoeba coli*, *Cryptosporidium sp.*, *Blastocystis sp.*, *Giardia lamblia*, *Ioadmoeba butschlii*, and *Endolimax nana*. Helminthic infections in this study were described as infections caused by *Ascaris lumbricoides*, *Trichuris trichiura*, *Hookworm*, dan *Hymenolepis nana*.

All research data were processed using statistical software. Descriptive data were presented in tabular form, and further analysis to examine the relationship between risk factors and protozoal or helminthic infections was conducted using the chi-square and Fisher's exact tests with a significance level of $P < 0.05$ and a 95% confidence interval.

Results

The study was conducted within an approximately 0.52 km² area (Figure 1). The population of this area primarily consists of small-scale pig farmers and waste pickers. The geographic boundaries of the study site are as follows: to the north, the study area is bordered by the Graha Metropolitan golf course; to the east, it extends to Sinurat Alley; to the south, it is bounded by the Village Hall Road; and to the west, it is limited by the entrance to the Medan-Binjai toll road.

Figure 1. Map of the study area

Certain areas within the research site lack adequate drainage systems. As a result, during rainfall, significant water accumulation occurs, often inundating residential properties (Figure 3). These conditions render the research site a high-risk environment for the transmission of intestinal parasites, particularly protozoa, among the local population.

A sampling process was conducted by identifying residents who were willing to have their homes serve as sample collection posts. Subsequently, an explanation was provided to the residents in the vicinity of these posts regarding the research pro

Figure 2. Actual view of several research locations

cedures, benefits, risks, and rights and obligations of participating in the study. This research yielded 7 residents who agreed to have their homes as sample collection posts, and 139 families who consented to participate in the study after being informed about the research. Furthermore, from the 139 families, 322 family members were found to meet the inclusion criteria and did not fall into the exclusion criteria. Subsequently, families who agreed were provided with stool specimen collection kits, and stool collection was conducted three times over a minimum of three consecutive days. Specimens were examined microscopically using two methods: direct smear with Lugol's iodine and acid-fast staining using the modified Kinyoun-Gabette method.

A total of 103 participants (31.99%) were aged 18-65 years, 103 (31.99%) had completed high school or equivalent education, and 136 (42.24%) were employed in various occupations, including 87 (63.97%) who were scavengers or livestock farmers. A significant proportion, 110 individuals (34.16%), reported a monthly household income of Rp. 1,000,000 - Rp 2,500,000, and 181 (56.21%) had 3-5 family members residing in the same household.

Subsequently, all participants underwent fecal laboratory examination. The analysis of human stool specimens revealed the presence of various intestinal parasites, including both helminths and protozoa (Table 2). Protozoan infections were detected in 113 specimens, with 86 cases of single infections and 27 instances of multiple protozoa. Helminth infections were found in 81 specimens, with 70 single infections and 11 cases of multiple helminths. *Ascaris lumbricoides* was the most prevalent helminth, identified in 53 individuals (16.46%), while *Entamoeba coli* was the most common protozoan, found in 41 participants (12.73%).

In addition to human fecal specimens, this study examined fecal specimens from domestic pigs owned by the study participants. Laboratory examination of 24 pig fecal specimens revealed the presence of various intestinal parasites (Table 3), with Isospora suis cysts being the most prevalent, detected in 18 samples (75%).

Table 1. Demographic distribution of subjects

Demographic	n	%
Age (years)		
0-5	42	13,04
5-<18	108	33,54
18-65	163	50,62
>65	9	2,80
Education		
No formal education	54	16,77
Elementary school	96	29,81
Middle school	62	19,26
High school	103	31,99
College	7	2,17
Occupation		
Unemployed	50	15,53
Student	106	32,92
Self-employed	25	7,76
Private employee	5	1,55
Other	136	42,24
Average monthly family income		
< Rp. 500.000	103	31,99
Rp. 500.000 – Rp. 1.000.000	97	30,12
Rp. 1.000.000 – Rp. 2.500.000	110	34,16
Rp. 2.500.000 – Rp. 5.000.000	12	3,73
Number of family members in one household		
<3	25	7,76
3-5	181	56,21
>5	116	36,03
Livestock Farming		
Yes	226	70,19
No	96	29,81

Table 2. Types of parasites in fecal specimens of study subjects

Parasite type	n	%
Helminth		
Ascaris lumbricoides	53	16,46
Trichuris trichiura	28	8,70
Hookworm	10	3,11
Hymenolepis nana	2	0,62
Protozoa		
Entamoeba coli	41	12,73
Cryptosporidium sp.	33	10,25
Blastocystis sp.	22	6,83
Giardia lamblia	21	6,52
Ioadmoeba butschlii	20	6,21
Endolimax nana	6	1,86

Table 3. Types of parasites in fecal specimens of pet pigs of study subjects

Parasite type	n	%
Helminth		
Ascaris suum	3	12,50
Hookworm	5	20,83
Protozoa		
Isospora suis	18	75,00
Balantidium coli	5	20,83
Giardia lamblia	1	4,17

The distribution of protozoan infections, based on specimen collection posts, revealed that the Pinggir Tol post exhibited the highest percentage of protozoan infections at both the family and individual levels (64.71% and 40%, respectively). Conversely, helminthiasis and combined intestinal parasitic infections were most prevalent at the Karya VII post, with percentages of 72.73% and 69.23% for helminthiasis, and 90.91% and 84.62% for combined intestinal parasitic infections at the family and individual levels, respectively (Table 4).

Table 4. Distribution of gastrointestinal parasite infections by specimen collection site

Post	Number of families	Indivi-duals	Protozoan infections		Helminth infections		Gastrointestinal parasite infections	
			Families	Individuals	Families	Individuals	Families	Individuals
Sempurna	30	73	12 (40,00)	21 (28,77)	9 (30,00)	14 (19,18)	14 (46,67)	30 (41,10)
Kemiri	15	27	9 (60,00)	10 (37,04)	1 (6,67)	1 (3,70)	9 (60,00)	11 (40,47)
Prasejahtera VII	18	48	10 (55,56)	19 (39,58)	7 (38,89)	12 (25,00)	14 (77,78)	27 (56,25)
Prasejahtera II	38	84	21 (55,26)	31 (36,90)	14 (36,84)	21 (25,00)	26 (68,42)	44 (52,38)
Karya VII	11	26	6 (54,55)	8 (30,77)	8 (72,73)	18 (69,23)	10 (90,91)	22 (84,62)
Prasejahtera IX	10	24	6 (60,00)	8 (33,33)	3 (30,00)	5 (20,83)	7 (70,00)	11 (45,83)
Pinggir Tol	17	40	11 (64,71)	16 (40,00)	9 (52,94)	10 (25,00)	13 (76,47)	20 (50,00)
Total	139	322	75 (53,96)	113 (35,09)	51 (36,69)	81 (25,16)	93 (66,91)	165 (51,24)

Statistical analysis of the research data (Table 5) revealed a significant association between age and Protozoa infection (p = 0.001), helminth infection (p = 0.0002), and overall intestinal parasitic infection (p = 0.0001). Conversely, several other variables showed no significant correlation with intestinal parasitic infections.

Table 6 shows that age was significantly associated with Protozoa infection (p = 0.001), helminth infection (p = 0.0002), and overall intestinal parasitic infection (p = 0.0001). However, other variables did not exhibit a significant association with intestinal parasitic infection. There was no significant correlation between levels of sanitation and hygiene and the prevalence of intestinal parasitic infections.

Discussion

In this study, *Entamoeba coli* infection was the most common infection found in stool specimen examinations, and *Ascaris lumbricoides* was the most prevalent worm species. These findings differ from those of Kache et al., who found hookworm to be the most common soil-transmitted helminth (10.9%), and Bahmani et al., who found *Blastocystis sp.* to be the most prevalent intestinal protozoa, accounting for 21.30% of all samples.[22,23]

Table 5. Association between risk factors and intestinal parasite infection among subjects

Risk factors	Protozoa infection			Helminth infection			Intestinal parasite infection		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p*
Age									
< 18 tahun	67(44,67)	83(55,33)	0,001	52(34,67)	98(65,33)	0,002	95(63,33)	55(36,67)	0,001
≥ 18 tahun	46(26,74)	126(73,26)		29(16,86)	143(83,14)		70(40,70)	102(59,30)	
Sex									
Male	44(40,00)	66(60,00)	0,018	30(27,27)	80(72,73)	0,528	60(54,55)	50(45,45)	0,393
Female	69(32,55)	143(67,45)		51(24,06)	161(75,94)		105(49,53)	107(50,47)	
Toilet									
Squat toilet	2(66,77)	1(33,33)	0,282*	1(33,33)	2(66,67)	1,000*	2(66,67)	1(33,33)	1,000*
Septic tank	111(34,80)	208(65,20)		80(25,08)	239(74,92)		163(51,10)	156(48,90)	
Pig farming									
Yes	80(35,40)	146(64,60)	0,860	59(26,11)	167(73,89)	0,546	119(52,65)	107(47,35)	0,437
No	33(34,38)	63(65,63)		22(22,92)	74(77,08)		46(47,92)	50(52,08)	
Frequent diarrhea									
Yes	33(38,37)	53(61,63)	0,457	21(24,42)	65(75,58)	0,854	46(53,49)	40(46,51)	0,626
No	80(33,90)	156(66,10)		60(25,42)	176(74,58)		119(50,42)	117(49,58)	
Diarrhea in the last week									
Yes	16(29,63)	38(70,37)	0,356	9(16,67)	45(83,33)	0,115	23(42,59)	31(57,41)	0,163
No	97(36,19)	171(63,81)		72(26,87)	196(73,13)		142(52,99)	126(47,01)	
Frequent abdominal pain									
Yes	29(31,52)	63(68,48)	0,396	20(21,74)	72(78,26)	0,397	41(44,57)	51(55,43)	0,130
No	84(36,52)	146(63,48)		61(26,52)	169(73,48)		124(53,91)	106(46,09)	

"Fisher's Exact test

In the examination of porcine fecal specimens, *Isospora suis* was the most prevalent parasite, detected in 75% of samples, followed by *Balantidium coli* at 20.83%. This finding contrasts with the study conducted by Symeonidou et al., which examined 1,150 fecal specimens from 8 intensive pig farms and reported *Balantidium coli* as the most common parasite (37.80%). However, this discrepancy may be attributed to the substantial variability in the prevalence of *Balantidium coli* among swine populations, as evidenced by the literature review conducted by Ahmed et al. spanning 1989 to 2019, which revealed a prevalence range of 0.70% to 100% across 27 studies. This wide variation can be attributed to several factors, including staining techniques, specimen preparation methods (direct smears versus concentration), and the intermittent nature of parasite shedding.[24,25] To address these potential biases, future studies should employ multiple staining methods and specimen preparation techniques, as well as increase the frequency of sample collection.

While *Balantidium coli* cysts were detected in porcine fecal samples, no such cysts were found in human fecal specimens. This discrepancy may be attributed to several factors, including: improved knowledge and practices among the population regarding swine husbandry, reduced pathogen virulence, or the possibility that the species identified in porcine feces was *Balantidium suis* or another species that is non-infectious to humans. To definitively determine the species of *Balantidium* observed in microscopic examinations, PCR analysis is recommended. Prior studies have reported similar findings, with high prevalence of *B. coli* in swine populations but without a corresponding increase in human infections. In a study conducted by Hajar S., while a 4.48% human infection rate was observed, the prevalence in swine populations (13.26%) remained undetermined.[13,26]

Microscopic examination of *Giardia lamblia* using Lugol's iodine wet mount demonstrated reasonably high sensitivity and specificity, at 85.7% (95% CI 62.6-96.2) and 100% (95% CI 96.3-100), respectively. Kinyoun-Gabette staining exhibited similar sensitivity and specificity of 85.70% (95% CI 56.2-97.5) and 100% (95% CI 96.5-100) in detecting *Cryptosporidium parvum* oocysts. A primary challenge in diagnosing *G. lamblia* infections is the intermittent nature of cyst shedding, while the small size of *C. parvum* oocysts (4-10 µm) poses a diagnostic hurdle. Although PCR is a highly sensitive method, a study by Elsafi et al. reported a notably lower specificity of 27% for this technique.[27]

Table 6. Association between sanitation and hygiene with intestinal parasite infection

Category	Intestinal parasite infection				p
	Positive		Negative		
	(n)	(%)	(n)	(%)	
Sanitation					
Poor	49	41,18	70	58,82	0,121
Moderate	32	28,32	81	71,68	
Good	32	35,56	58	64,44	
Hygiene					
Poor	20	83,33	4	16,67	0,155
Moderate	78	63,41	45	36,59	
Good	111	63,43	64	36,57	

The correlation between community sanitation levels and the incidence of intestinal parasitic infections yielded a p-value of 0.121, which is greater than the significance level of 0.05. This indicates that there is no significant association between these two variables.

Similarly, the relationship between personal hygiene levels and the incidence of intestinal parasitic infections resulted in a p-value of 0.155, also exceeding the 0.05 threshold. This further confirms the absence of a significant correlation between these variables. Laboratory examinations revealed the presence of *Hymenolepis nana* eggs in two specimens (0.62%). Human infections with this tapeworm are relatively uncommon, and the two positive specimens were collected from distinct locations: Karya VII and Pinggir Tol.

Conclusion

Based on the research findings and discussion, there was no statistically significant relationship between the risk factors and intestinal protozoa infection. However, this study indicates that intestinal parasite infections, especially protozoa, are still prevalent in the community, both symptomatic and asymptomatic. Further research is needed to elucidate the complex interplay of factors contributing to the persistence of these infections in the region.

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