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Original Article

Risk Factor of *Blastocystis hominis* and *Giardia duodenalis* among Stunted Children in Bandung Regency, Indonesia

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Abstract

Background: Stunting, resulting from chronic malnutrition, increases susceptibility to infections due to immature immunity. *Blastocystis hominis* and *Giardia duodenalis* may contribute to stunting. We aimed to determine the characteristics of intestinal protozoan infection among stunting children.

***Methods*:** A cross-sectional study was conducted in 2020 among 280 stunted children in Bandung Regency, West Java, Indonesia. Faecal specimens were collected, with portions preserved separately in 10% formaldehyde and RNA Later solution. Of these, 230 met the examination criteria. Risk factors and demographic data were obtained through interviews. DNA was extracted, and intestinal protozoan infection were detected using PCR targeting the 18S SSU rRNA gene for *B. hominis* and 16S-like RNA gene for *G. duodenalis*.

***Results*:** The prevalence of *G. duodenalis* and *B. hominis* was 5.6% (13/230) and 55.6% (128/230), respectively. Multivariate analysis identified age (*P-value* 0.004; OR 0.327) and no availability of a septic tank (*P-value* 0.021; OR 4.881) were the significant risk factors for *G. duodenalis* infection. For *B. hominis* infection, significant risk factors included age (*P-value* 0.033; OR 0.722) and gender (*P-value* 0.047; OR 1.742).

***Conclusion*:** Stunting and intestinal protozoan infection present a dual burden. *G. duodenalis* and *B. hominis* infections were prevalent among stunted children. Significant risk factors included age and septic tank unavailability for *G. duodenalis*, while age and gender were associated with *B. hominis* infection. Improved sanitation and targeted interventions are essential to reduce infection risks.

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Introduction

Blastocystis hominis (B. hominis) and *Giardia duodenalis* are intestinal protozoa that pose significant public health concerns (1). Globally, intestinal protozoan infections affect approximately 3.5 billion people, leading 450 million symptomatic cases and 200,000 deaths annually (2). These two protozoans are among the most common causes of gastrointestinal illness in children (3). Due to their immature immune systems, children are particularly vulnerable, with risks further exacerbated by exposure to poor personal hygiene and a lack of health education (4). While school-aged children have a higher reported burden, Children under five also face considerable risk (5). Their developing immunity, poor nutritional intake, and frequent hand-to-mouth behaviors contribute to increased infection susceptibility (6).

Stunting, caused by chronic malnutrition, weakens immunity, and heightens susceptibility to infections, including *B. hominis* and *G. duodenalis* (6). Moreover, repeated parasitic infections can worsen malnutrition by causing nutrient malabsorption, which further perpetuates the cycle of stunting and poor health (7). Studies show that stunted children are more prone to parasitic infections, which further delay their cognitive and physical development (6). Since these infections often cause chronic diarrhea and gut inflammation, understanding their prevalence in stunted populations is essential for developing targeted health interventions.

B. hominis and *G. duodenalis* were selected for this study due to their significant public health impact and potential role in exacerbating malnutrition and stunting. *G. duodenalis* is a well-documented cause of persistent diarrheal and malabsorption, particularly in children living in low-resource settings with poor sanitation (8). *B. hominis*, despite its high global prevalence, remains clinical controversial, though increasing evidence suggests its role in chronic diarrhea, gut microbiota imbalance, and intestinal inflammation (9). Both parasites are associated with poor hygiene, contaminated water, and inadequate sanitation—factors commonly found in regions with high stunting rates. Investigating their prevalence and risk factors in stunted children can clarify their contribution to the stunting-malnutrition-infection cycle and informs targeted intervention.

In immunocompromised individuals, including stunted children, *B. hominis* and *G. duodenalis* infections can cause severe morbidity, anorexia, malabsorption, and cognitive decline (10). Prolonged stunting and parasitic infections may lead to chronic disease (11) and future malnutrition (12). Studies have shown an increased infection rate of *B. hominis* and *G. duodenalis* in stunted children (13) and a higher prevalence of stunting among infected individuals (14). Various factors influence infection, including pathogens, reservoir, transmission routes, and host susceptibility (15). Identifying the risk factors for intestinal protozoan infections is crucial for designing effective prevention strategies (16).

West Java Province has one of Indonesia's highest stunting rates at 31.1% (17). In response, ten villages have been prioritized for stunting control program. Additionally, only 39.8% of Bandung Regency's Population practice proper hygiene, yet data on the prevalence of *B. hominis* and *G. duodenalis* infections remains unavailable. Understanding the risk factors influencing these infections is essential for designing effective intervention strategies, as risk may vary across regions. This study aimed to determine the characteristics and risk factors of *B. hominis* and *G. duodenalis* infections in stunted children, contributing to policies for managing intestinal protozoan infections in affected communities.

Materials and Methods

Ethics Approval

The study was conducted following the Declaration of Helsinki and received approval from the Research Ethics Commission of

Universitas Padjadjaran (Code: 825/UN6.KEP/EC/2021).

Study Design

A cross-sectional study was conducted between January and March 2020 across 11 districts in Bandung Regency, West Java, Indonesia (Fig. 1). Laboratory analyses were performed at the Laboratory of Parasitology, Faculty of Medicine, Universitas Padjadjaran.

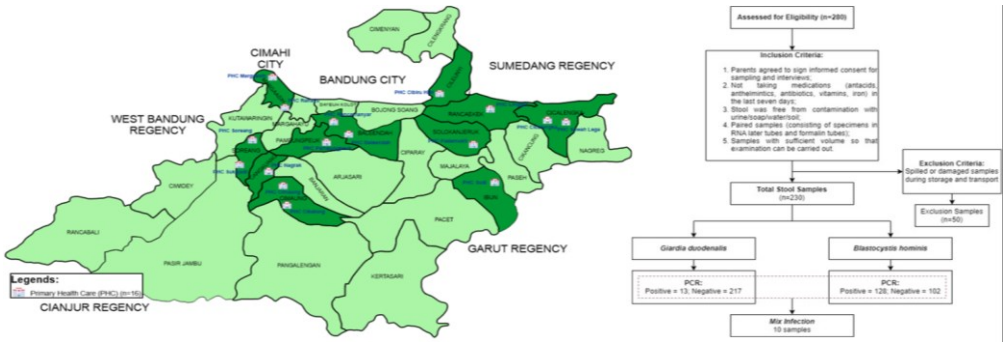

Fig. 1: The study covered 16 Primary Health Centers (*Puskesmas*) in 11 districts. A total of 280 samples were collected, of which 230 met the eligibility criteria and were included in the analysis

Study Population and Sampling

This study targeted stunted children aged 3-6 years registered at the Bandung Regency District Health Office. The study flow chart is shown in Fig. 1. A stratified random sampling method selected 280 participants based on the following inclusion criteria: 1) parents agreed to sign informed consent for sampling and interviews; 2) not taking medications (antacids, anthelmintics, antibiotics, vitamins, iron) in the last seven days; 3) stool was free from contamination with urine/soap/water/soil; 4) paired samples (specimens in RNA later and formalin preserved); 5) sufficient volume. Exclusion criteria included spilled or damaged samples during storage and transport. After applying these criteria, 230 stool samples were analyzed.

Data Collection

Demographics and risk factors data were obtained through structured interviews with parents or caregivers, covering age, gender, parental education (low education for not attending school up to junior high school, and higher education for senior high school levels up to diploma/bachelor), income (based on regional wage standards), water source (well or treatment water), distance from water source to the septic tank (<10 m or >10 m), septic tank availability, and hand hygiene.

Stool Sample Collection and Preservation

Fecal samples were collected from selected children, with parents/guardians instructed on proper collection. Each sample was divided into two portions, preserved in 10% formaldehyde and RNA Later solution (RNA*later*™ RNA Stabilization Reagent, Lot. 145047517). Although primarily designed for RNA preservation RNA Later effectively stabilizes DNA, preventing degradation during storage and transport. This ensured DNA integrity for subsequent PCR amplification of the 16S or 18S rDNA genes (18). Samples were stored at -20 °C and -80 °C. PCR was used to detect *G. duodenalis* and *B. hominis*.

DNA Extraction

DNA from *Giardia sp.* and *Blastocystis sp.* was isolated according to the procedure of the QIAmp® Fast DNA Stool Mini Kit (Qiagen QIAmp® Fast DNA Stool Mini Kit, Lot. 166031190) from 200 mg faecal sample, which

had undergone pre-treatment: manual grinding with a pestle and 20 cycles of freeze-thawing in liquid nitrogen and 70 °C water bath.

PCR Detection

The isolated DNA was then subjected to PCR examination using primers targeting 600 bp, using the primers RD5 (5'- ATC TGG TTG ATC CTG CCA GT -3' and BhRDr (5'-GAG CTT TTT AAC TGC AAC AAC G-3') for *B. hominis* (19), and 16S like RNA (nested 1 giard-18-fwd: 5'- TCA ACG TYA AYC GYG GYT TCC GT - 3'; nested 1 giard-18-rev: 5'- GTT RTC CTT GCA CAT CTC C - 3'; nested 2 giard-18-fwd: 5' - CAG TAC AAC TCY GCT CTC GG -3'; and nested 2 giard-18-rev: 5' - GTT RTC CTT GCA CAT CTC C - 3') targeting 432 bp for *G. duodenalis* (20).

The PCR condition for *B. hominis* was started with primary denaturation at 95 °C for 5 minutes, followed by 30 cycles including denaturation at 94 °C for 30 seconds, annealing at 59 °C for 30 seconds, extension at 72 °C for 30 seconds, with a final elongation at 72 °C for 10 minutes (19). The PCR condition for *G. duodenalis* was carried out with primary denaturation at 95 °C for 5 minutes, followed by 40 cycles of 45 seconds each at 95 °C, annealing at 60 °C for 30 seconds, extension at 72 °C for 45 seconds, with a final elongation at 72 °C for 7 minutes and 1 minute at 60 °C (20). The PCR product was electrophoresed at 100 Volt for 30 minutes on 1.5% agarose gel stained with GelGreenTM (Mini Bio, GelGreen™ Nucleic Acid Stain, Lot. 20G021-2105). The amplicon was visualized on a UV transilluminator. The final diagnosis of *B. hominis* and *G. duodenalis* infections was based on the PCR results.

Statistical Analysis

Statistical analysis was performed using STATA Special Edition 17th Version. Bivariate analysis was conducted using the Chi-Square test. Multivariate analysis was performed using multiple logistic regression to determine the most influential risk factors for *B. hominis* and *G. duodenalis* infection, with a significance level of 5% (*P-value* <0.05).

Results

PCR Result

PCR analysis was conducted on 230 stool samples to detect intestinal protozoan infections and associated risk factors. Results showed that 128 samples (55.6%) were positive for *B. hominis* and 13 samples (5.6%) were positive for *G. duodenalis*. Additionally, 10 samples exhibited mixed infections with both protozoa. The presence of distinct DNA bands confirmed successful amplification (Fig. 2).

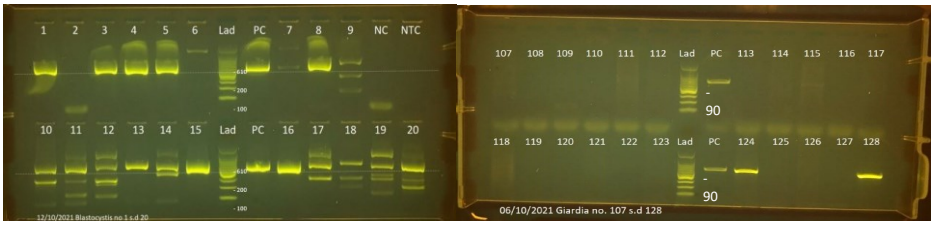

Fig. 2: Agarose gel electrophoresis of *B. hominis* (left) and *G. duodenalis* (right). The 610 bp PCR amplicons of *B. hominis* and the 900 bp PCR amplicons *of G. duodenalis* are shown

Distribution of Intestinal Protozoan Infections

Figure 3 shows the distribution of intestinal protozoan infections across 11 districts and 16 Primary Health Centers (PHCs) in Bandung Regency. *B. hominis* or *G. duodenalis*, or both were detected in all. Pameungpeuk District had the highest infections (20 cases of *B. hominis* and 3 cases of *G. duodenalis*), while Cileunyi District had the lowest (3 cases of *B. hominis*).

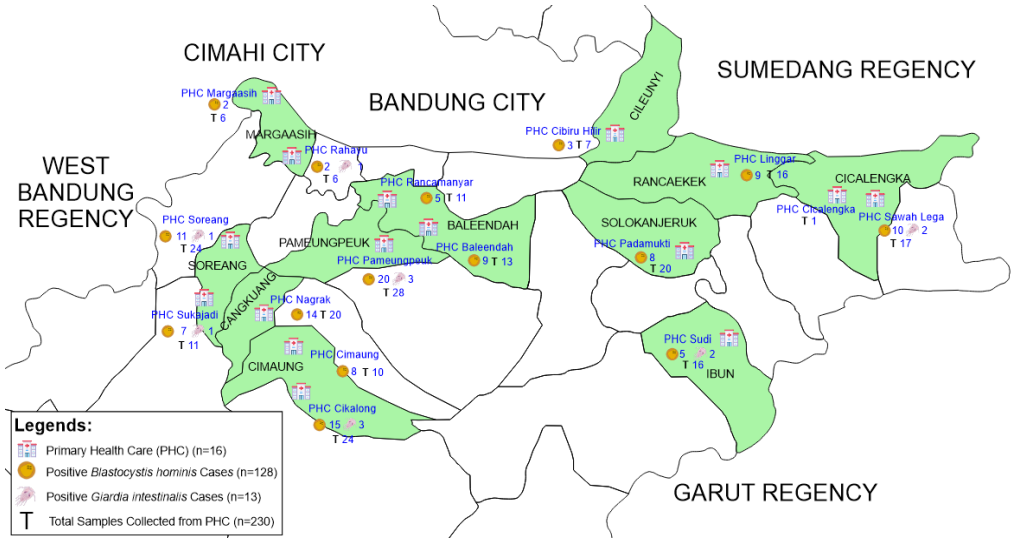

Fig. 3: Distribution of Intestinal Protozoan Infection in Bandung Regency

Bivariate Analysis

Table 1 present the bivariate analysis of demographic, socioeconomic, and environmental factors influencing *G. duodenalis* and *B. hominis* infections in stunted children. Significant risk factor included gender, males had higher infection rate with *G. duodenalis* (*P-value* = 0.039, OR = 3.689) and *B. hominis* (*P-value* = 0.016, OR = 1.913). Additionally, septic tank availability was significantly associated with *G. duodenalis* infection (*P-value* = 0.024, OR = 3.613), suggesting an environmental impact on transmission. Other factors, such as parental education, income, water source, and hand hygiene, were not significant associations.

Table 1: Bivariate Analysis based on Characteristics and Risk Factors

Variable	Giardia duodenalis		*P*-value	Odds Ratio (95% CI)	Blastocystis hominis		*P*-value	Odds Ratio (95% CI)
	Positive	Negative			Positive	Negative		
	n= 13 (%)	n=217 (%)			(n=128)	(n=102)		
Age (year± st. dev)	(5 ± 1)	(4 ± 1)		n/a	(5 ± 1)	(4 ± 1)		n/a
Gender								
Male	10 (8.8)	103 (91.2)	0.039*	3.689	72 (63.7)	41 (36.3)	0.016*	1.913
Female	3 (2.6)	114 (97.4)		(0.988 – 1.775)	56 (47.9)	61 (52.1)		(1.128 – 3.243)
Father's Education								
Low Education	11 (6.5)	159 (93.5)	0.366	2.006	100 (58.8)	70 (41.2)	0.103	1.633
High Education	2 (3.3)	58 (96.7)		(0.432 – 9.324)	28 (46.7)	32 (53.3)		(0.903 – 2.951)
Mother's Education								
Low Education	12 (6.9)	162 (93.1)	0.150	4.074	101 (58.0)	73 (42.0)	0.198	1.486
High Education	1 (1.8)	55 (98.2)		(0.518 – 32.055)	27 (48.2)	29 (51.8)		(0.812 – 2.720)
Incomes								
Below the minimum wage	13 (6.1)	200 (93.9)	0.294	n/a	118 (55.3)	95 (44.7)	0.784	0.869
Above the minimum wage	0 (0)	17 (100)			10 (58.8)	7 (41.2)		(0.319 – 2.370)
Water Source								
Well	9 (4.7)	183 (95.3)	0.154	0.418	109	83 (43.2)	0.433	1.313

Table 1: Continued…

					(56.8)			
Treatment Water	4 (10.5)	34 (89.5)		(0.122 – 1.435)	19 (50)	19 (50)		(0.654 – 2.637)
Distance from water source to Septic tank								
< 10m	9 (5.56)	153 (94.44)	0.922	0.941	96 (58.3)	66 (40.7)	0.089	1.636
> 10m	4 (5.89)	64 (94.11)		(0.280 – 3.167)	32 (47.1)	36 (52.9)		(0.925 – 2.894)
Septic Tank Availability								
No	5 (13.5)	32 (86.5)	0.024*	3.613	19 (51.4)	18 (48.6)	0.565	0.813
Yes	8 (4.1)	185 (95.9)		(1.112 – 11.743)	109 (56.5)	84 (43.5)		(0.402 – 1.646)
Hand Hygiene								
No	2 (5.1)	37 (95.9)	0.876	0.885	17 (43.6)	22 (56.4)	0.096	0.557
Yes	11 (5.8)	180 (94.2)		(0.188 – 4.157)	111 (58.1)	80 (41.9)		(0.278 – 1.116)

Note: *=significant (*P-value* <0.05)

Multivariate Analysis

Table 2 presents a multivariate logistic regression assessing risk factors for *G. duodenalis* and *B. hominis* infections. Significant predictors for *G. duodenalis* infection included age (*P-value* = 0.004, OR = 0.327), with younger children at higher risk, and septic tank unavailability (*P-value* = 0.021, OR = 4.881), indicating poor sanitation increased risk of infection.

For *B. hominis*, significant factors were age (*P-value* = 0.033, OR = 0.722), where risk decreased with age, and gender (*P-value* = 0.047, OR = 1.742), with males more susceptible. Other variables, including parental education, water source, and distance to the septic tank, were not significantly associated.

Table 2: Multivariate Analysis of Risk Factors

Variable	*Giardia duodenalis*		*Blastocystis hominis*	
	P-value	**Odds Ratio (95% CI)**	**P-value**	**Odds Ratio (95% CI)**
Age	0.004*	0.327 (0.153 – 0.700)	0.033*	0.722 (0.536 – 0.974)
Gender	0.074	3.741 (0.881 – 15.886)	0.047*	1.742 (1.006 – 3.016)
Father's Education	0.766	0.763 (0.128 – 4.545)	0.571	1.258 (0.568 – 2.785)
Mother's Education	0.235	4.063 (0.403 – 41.015)	0.688	1.181 (0.525 – 2.656)
Water source	0.335	0.506 (0.127 – 2.019)	0.342	1.427 (0.686 – 2.970)
No availability Septic Tank	0.021*	4.881 (1.274 – 18.699)	0.582	0.813 (0.389 – 1.700)
Distance from water source to Septic Tank	0.713	0.779 (0.206 – 2.942)	0.111	1.613 (0.897 – 2.903)

Note: *=significant (*P-value* <0.05)

Discussion

This study highlights the high prevalence of intestinal protozoan infection among stunted children in Bandung Regency, West Java, indicating a double burden of stunting and infection. These findings emphasize the urgent need for immediate intervention. Intestinal protozoan infections are particularly prevalent

in low-income regions with poor hygiene (21), further exacerbating children's nutritional deficiencies (22). The impact of these infections on stunting is influenced by several factors, including infection level, type, time, duration, intensity, and pathogenicity. Multiple mechanisms contribute to this process, such as reduced nutrient intake, diarrhoea, environmental enteric dysfunction, gut microbiota interaction, chronic immune activation, systemic inflammation, anaemia, and epigenetic regulation alteration (23).

The PCR analysis revealed that 5.6% of children were infected with *G. duodenalis*, similar to report from India (4.9%) (24), Mexico (7.9%) (25), and Southern Ethiopia (10.45%). However, this prevalence was significantly lower compared to studies in Indonesia, such as East Nusa Tenggara Province (19.0%) (26) and South Sumatra Province (37.1%) (27). Meanwhile, *B. hominis* infection was detected in 55.6% of cases, consistent with findings in Argentina (42.2%) (28) and Nicaragua (48.6%) (29) but much higher than in Pakistan (1.8%) (30) and Iraq (4.6%) (31). Compared to previous studies in Indonesia, the prevalence in this study was higher than that reported in East Nusa Tenggara Province (34.5%) (14) and in three other Provinces (Jakarta, South Kalimantan, and South Sulawesi), which reported a prevalence of 12.46% (32).

Multivariate analysis identified age as a key risk factor for protozoan infections. In Turkey, children older than 37 months had a 1.93 times higher risk of intestinal protozoan infection than younger children ($p < 0.05$) (33). In Rwanda, *G. duodenalis* infection peaked in youngest school-aged children (above 70%) and gradually declined with age, though prevalence remained above 20% in teenagers. These findings highlight the significant role of age in the susceptibility to *G. duodenalis* infection (34). As children grow, increased physical activity and outdoor play raise exposure risk (35). Younger children receive better protection through breastfeeding and limited environmental exposure (36). However, other studies associate higher infection risk in younger children with poor toilet training, hygiene, low socioeconomic status, overcrowding, and daycare exposure (37). Some studies, however found no significant association between age and infection risk (38,39).

Gender was a significant risk factor for *B. hominis* infection, with males having almost twice the risk of females. This contrasts with studies in Nicaragua and Malaysia, where females had higher, though the statistically insignificant (29,40). Other studies in Europe have also reported no gender differences. However, research from Asia and Africa suggests females may be at higher risk due to dietary habits (39). *Blastocystis sp.* infection has been linked to stunting, impaired growth, and cognitive deficits (41), though its exact impact on nutrition remains unclear.

Another key finding was that the absence of a septic tank increased *G. duodenalis* infection fivefold. In East Nusa Tenggara, lack of septic tank was associated with a fourfold higher risk (42), and in Southern Ethiopia, poor sanitation doubled infection risk (14). A systematic review confirmed that inadequate toilets contribute to *G. duodenalis* transmission via contaminated water, soil, and plants (37).

This study has several limitations that should be acknowledged. Firstly, data collection was conducted at a single time point, which limits the ability to assess causal or long-term relationships between stunting and intestinal protozoan infections. Additionally, there was a considerable time gap between data collection and manuscript submission, primarily due to the extended data verification process and disruptions caused by the COVID-19 pandemic, which significantly affected laboratory operations and research workflows. Despite these limitations, the findings offer valuable insights into demographic trends and key risk factors, and the continued relevance of protozoan infections among stunted children underscores the importance of this study as a foundation

for future public health research and interventions.

Conclusion

This study highlights the dual burden of stunting and intestinal protozoan infections. *G. duodenalis* and *B. hominis* were detected in 5.6% (13/230) and 55.6% (128/230) of cases, respectively. Age and lack of a septic tank were significant risk factors for *G. duodenalis*, while age and gender were associated with *B. hominis* infection.

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Conflict of Interest

The authors declare no conflict of interest.

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