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Prevalence and Risk Factors of *Giardia intestinalis* Infection among Patients in Laghman and Nangarhar Provinces in Eastern Afghanistan

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ABSTRACT

Background: *Giardia intestinalis*, a gastrointestinal protozoan parasite, is a significant cause of diarrhea and abdominal discomfort, particularly in developing countries. We aimed to determine the prevalence and risk factors associated with *G. intestinalis* in Laghman and Nangarhar provinces in Eastern Afghanistan from January to December 2023.

Methods: A cross-sectional study was conducted among 1,351 patients with gastrointestinal symptoms, including 604 from Laghman and 747 from Nangarhar provinces, Afghanistan. Stool samples were collected from each participant using sterile containers and subsequently analyzed under light microscopy to identify the presence of *G. intestinalis* cysts or trophozoites. Individuals who tested positive for *G. intestinalis* were identified as cases. An equal number of *Giardia*-negative individuals were selected randomly as controls for risk factor analysis. All participants identified as *Giardia*-positive completed a structured questionnaire to collect data on demographic, socioeconomic, environmental, behavioral, seasonal, and clinical factors. Statistical analyses were conducted to determine significant risk factors.

Results: Overall, 124 samples (9.2%) tested positive for *G. intestinalis*, with positivity rates of 7.5% in Laghman and 10.6% in Nangarhar. In Laghman, male sex, domestic animal ownership, soil contact, swimming in unregulated water bodies, and consumption of unfiltered water were significantly associated with infection (P<0.05). In Nangarhar, female sex and higher monthly income were identified as significant risk factors. The most commonly reported symptoms among positive cases were abdominal pain and diarrhea. Children aged 5-14 exhibited the highest positivity rates.

Conclusion: The findings indicate a notable prevalence of giardiasis among symptomatic patients in Laghman and Nangarhar, with a higher burden in Nangarhar. Various demographic, socioeconomic, environmental, and behavioral factors were identified as significant contributors to infection risk.

Keywords: Giardiasis, *Giardia intestinalis*, Middle East, Parasite, Parasitology, Prevalence, Public health, Risk factors.

Introduction

Gastrointestinal parasite (GP) infections represent a significant global health burden, particularly in underprivileged communities within tropical and subtropical regions (1).

Among these infections is giardiasis, caused by *Giardia intestinalis* (also referred to as *G. lamblia* or *G. duodenalis*), a flagellated protozoan that infects around 180 million individuals annually (2, 3). The infection is primarily transmitted through the fecal-oral route by ingesting water, food, or vegetables contaminated with *G. intestinalis* cysts, making it more common in developing countries due to inadequate sanitation, limited access to healthcare facilities, and poor-quality drinking water (4).

Individuals infected with *G. intestinalis* may be asymptomatic carriers or experience acute symptoms such as diarrhea, abdominal discomfort, nausea, and vomiting. If left untreated, the infection can become chronic, leading to malabsorption, which may result in nutrient deficiencies, anemia, growth retardation, and weight loss (5, 6). Post-infectious complications of giardiasis include irritable bowel syndrome (7), chronic fatigue syndrome (8), arthritis, allergies, cognitive impairment, and other extra-intestinal conditions (9).

While giardiasis is reported globally, it is notably more prevalent in developing countries, with positivity rates ranging from 20% to 30%, compared with rates of 2% to 5% in developed countries (10). A systematic review and meta-analysis assessed the prevalence of *G. intestinalis* among African children and estimated a pooled prevalence of 18.3%, with the highest and lowest rates in Niger and Cameroon, respectively (11). In a similar study focusing on Asian children, Kalavani et al. reported a pooled prevalence of 15.1%, with the highest rates in Tajikistan and Malaysia and the lowest in China and Saudi Arabia (4).

In Afghanistan, the pooled prevalence of *G. intestinalis* is 13.92%, and the reported prevalence varies considerably across regions and population groups (Table 1).

Among children, positivity rates range from 5.1% to 31.1% (12-18), while in adults, rates range from 2.7% to 10.1% by light microscopy, with up to 18.1% detected by antigen-ELISA testing (15, 18, 19). Among symptomatic individuals, positivity rates range from 5.1% to 17.2% (15, 17, 18, 20, 21). A study of asymptomatic Polish soldiers at the Forward Operating Base Ghazni revealed 2.7% prevalence by light microscopy and 18.1% by ELISA (19). Although males generally exhibit higher positivity rates, one study in Parwan found that adult females had a higher prevalence (15, 18, 20, 21). These findings underscore the variability in *G. intestinalis* prevalence, influenced by age, sex, and diagnostic methods.

Giardiasis remains a significant health concern among Afghan refugees and travelers to endemic regions. A study in the Mianwali district of Pakistan among Afghan refugees reported a 7.7% positivity rate (22), while Heravi et al. found an 8.8% prevalence among Afghan children in Kashan City, Iran (23). Higher prevalence rates were observed in Afghan refugees in camps in Pakistan before relocation to Sweden (24), and *G. intestinalis* was the most common infectious disease among newly arrived Afghan refugees in rural Australia (25). 10.3% prevalence was reported in children and 11.6% in adolescents among Afghan refugees resettled in Calgary, Canada (26).

Table 1: Prevalence of Giardiasis in Afghanistan and Among Afghan Refugee Populations

Ref. no.	***Author(s)***	***Study Period***	***Year of Publication***	***Province/Country***	***Sample Demographics***	***Symptomatic /Asymptomatic***	***Diagnostic Method***	***Sample Size (N)***	***Prevalence (%) of G. intestinalis***
(20)	Asad et al.	Jan – Dec 2023	2024	Wardak, Afghanistan	General population	Symptomatic	Light microscopy	274	6.9%
(13)	Rahimi et al.	May – Dec 2022	2023	Kandahar, Afghanistan	Primary School Children	No relevant data	Light microscopy	1,275	22.4%
(21)	Nekmal and Jan	Mar – Aug 2022	2023	Khost, Afghanistan	General Population	Symptomatic	Light microscopy	368	9.2%
(12)	Rahimi et al.	June – Oct 2020	2022	Kandahar, Afghanistan	Children	No relevant data	Light microscopy	1,426	13.9%
(14)	Lass et. al	Nov 2013 – April 2014	2017	Ghazni, Afghanistan	Children aged 7 – 18	No relevant data	Real-time PCR	245	21.2%
(15)	Korzeniewski et al.	Mar – Apr 2013 Oct – Nov 2014	2017	Ghazni, Afghanistan Parwan, Afghanistan	General Population	Symptomatic	Light microscopy	386 (179 children; 207 adults) 162 (93 children; 69 adults)	14.5% Children; 7.2% Adults 17.2% Children; 10.1% Adults
(16)	Korzeniewski et al.	Nov 2013 – April 2014	2016	Ghazni, Afghanistan	Children aged 7 - 18	No relevant data	Light microscopy	500	31.1%
(19)	Korzeniewski et al.	Aug 2011	2016	Ghazni, Afghanistan	Polish Soldiers (aged 22 – 48)	Asymptomatic	Light microscopy; RIDA Quick *Giardia* IC test; RIDA screen *Giardia* IE test	630	2.7% (by microscopy); 18.1% (by ELISA)
(17)	Elyan et al.	2009 - 2010	2014	Kabul & Kandahar, Afghanistan	Children < 5	Symptomatic	ELISA	699	5.1% (single infection) 12.1 % (single and mixed infection)
(18)	Tariq	Jan 2008 – June 2012	2013	Kabul, Afghanistan	General Population	Symptomatic	Light microscopy	20,040	9.0%
(26)	Smathi et al.	Jan 2011 – Dec 2020	2024	Calgary, Canada	Newly Resettled Afghan Refugees	Symptomatic	Laboratory methods (unspecified)	402	10.3% in children; 11.6% in adults
(22)	Haq et al.	Feb 2007 – Dec 2009	2015	Mianwali, Pakistan	Afghan refugees	Symptomatic	Light microscopy	687	37.7%
(23)	Heravi et al.	2009 - 2010	2013	Kashan, Iran	Afghan Children in Primary and Junior High Schools	No relevant data	Light microscopy	430	8.8%
(25)	Sanati et al.	April 2010 – March 2013	2014	Mildura Victoria, Australia	Afghan Refugees	No relevant data	Light microscopy	92	11.2%
(24)	Ekdahl and Andersson	1997 - 2003	2005	Sweden	International travelers, immigrants/refugees, and adopted children	No relevant data	Laboratory methods (unspecified)	Not applicable	3.8% (Afghan refugees)

CS = cross-sectional; IC = Immunochromatographic; IE = Immunoenzymatic; OS = Observational Study; RCS = retrospective cohort study

The differing prevalence of giardiasis across various countries and regions highlights the necessity for more in-depth research. Afghanistan has 34 provinces, but the studies conducted have been limited to only a few provinces: Kabul, Kandahar, Khost, Wardak, Parwan, and Ghazni (12-16, 20, 21).

We aimed to address this gap by estimating the prevalence of *G. intestinalis* by collecting data on positivity rates and identifying its associated risk factors in the provinces of Laghman and Nangarhar in Eastern Afghanistan.

Materials and Methods

Study area, population, and sample collection

This study was conducted from January to December 2023 in the eastern Afghan provinces of Laghman and Nangarhar, which cover approximately 2,790 and 3,060 square miles, respectively ((1), 28). According to the National Statistics and Information Authority (NSIA), the estimated populations in 2023 were 519,842 for Laghman and 1,805,087 for Nangarhar, with approximately equal sex distributions in both provinces. Children aged 0–14 comprised 52% of the population in Laghman and 51% in Nangarhar, and most residents in both provinces live in rural areas (29).

We conducted a cross-sectional study among 1,351 individuals (604 from Laghman and 747 from Nangarhar) enrolled passively as they presented with gastrointestinal symptoms. Stool samples were collected using sterile, labeled containers and microscopically examined for the presence of *G. intestinalis*. Individuals with confirmed infections were identified as cases, and an equal number of control participants were randomly selected from among the Giardia-negative individuals.

Questionnaire

In addition to stool sample testing, participants completed a structured questionnaire to collect data on potential risk factors. Demographic variables included age (categorized into five groups) and sex. Socioeconomic status included monthly household income, classified as low (<AFN 10,000), middle (AFN 10,000–20,000), and high (>AFN 20,000) based on the national income averages in Afghanistan. Clinical symptoms recorded included diarrhea (defined as the passage of three or more loose stools in 24 h) (30), as well as nausea, vomiting, abdominal pain, weakness, headache, and fever. Environmental factors encompassed domestic animal ownership, soil contact, and types of drinking water. Behavioral factors included recent travel history and swimming in unregulated water bodies. Lastly, we included temporal factors to assess which season had the most onsets of symptoms.

Statistical analysis

Data were analyzed using IBM SPSS Statistics ver. 26 (IBM Corp., Armonk, NY, USA). Separate analyses were conducted for each province to identify associated risk factors, followed by comparative analyses between Laghman and Nangarhar. Logistic regression was applied to determine whether age and sex were independently associated with *G. intestinalis* positivity. The model adjusted for these variables to account for potential confounding effects. A P-value<0.05 was considered statistically significant.

Ethical considerations

The Biomedical Ethics Committee of Ghalib University, Kabul, Afghanistan (AF.GUK.REC.1401.008) reviewed and approved the study protocol. Each participant provided informed written or oral (if illiterate) consent, covering participation in the physical examination and completing the questionnaires. For child participants, written consent was obtained from a parent or legal guardian. To protect privacy, all data were anonymized using unique participation codes and handled with strict confidentiality throughout the study.

Results

Positivity rates of G. intestinalis in Laghman and Nangarhar provinces

Overall, 1351 stool samples were collected: 604 from Laghman (311 males [51.5%], 293 females [48.5%]) and 747 from Nangarhar (436 males [58.3%], 311 females [41.6%]). Overall, 124 samples tested positive for *G. intestinalis*: 45 (7.5%) in Laghman and 79 (10.6%) in Nangarhar.

G. intestinalis and associated risk factors in Laghman

The distribution of positive cases across age groups and sex in Laghman, as shown in (Figure 1), indicated higher positivity rates among children aged 5 to 14. Males exhibited higher positivity rates than females across most age groups, as confirmed by chi-square analysis (*P*=0.032). Logistic regression analysis further confirmed that sex remained a significant predictor of positivity (*P*=0.02), while age showed no significant effect (*P*=0.564).

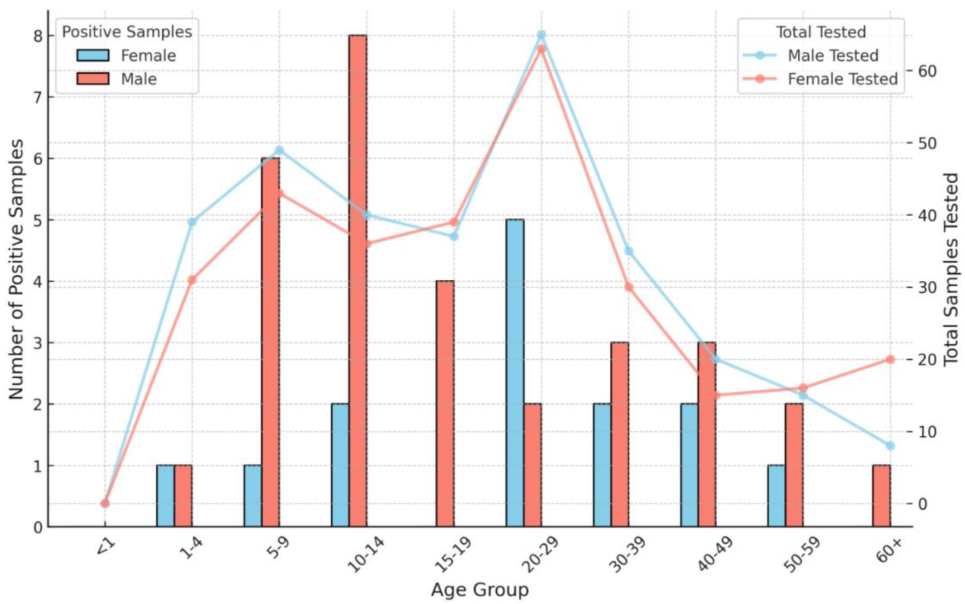

Figure 1: Distribution of G. intestinalis positive cases across age groups, stratified by sex, in Laghman province. The number of positive samples is shown as bars (blue for females, red for males), while the total number of individuals tested per group is represented by lines. Positivity was higher among children aged 5–14 yr compared to other age groups. Males had a significantly higher infection rate than females (*P*=0.032). Logistic regression analysis confirmed sex as a significant predictor of infection (P = 0.02), whereas age group was not statistically significant (*P*=0.564).

Several environmental and behavioral factors were significantly associated with *G. intestinalis* infection in Laghman (Table 2), including domestic animal ownership (*P*<0.0001), consumption of unfiltered water (*P*=0.001), soil contact (*P*=0.042), and swimming in unregulated water bodies (*P*=0.017). Infections peaked during autumn (*P*=0.016), followed by summer, spring, and winter (Table 3).

There were no significant associations with travel history or socioeconomic status; however, lower-income individuals tended to show higher positivity, though this trend was not statistically significant.

Table 2: Distribution of *G. intestinalis* in Laghman in relation to demographic, socioeconomic, environmental, and behavioral factors

Risk Factors	*Case (N = 45) n (%)*	*Controls (N = 45) n (%)*	χ^2	*P-value*
Socioeconomic				
Monthly income				
<10,000 AFN	26 (57.8)	24 (53.3)	1.080	0.583
10,000 – 20,000 AFN	14 (31.1)	18 (40.0)		
>20,000 AFN	5 (11.1)	3 (6.7)		
Environmental and Behavioral Factors				
Domestic animal ownership				
Yes	36 (80.0)	4 (8.9)	46.080	< 0.0001
No	9(20.0)	41(91.1)		
Source of drinking water				
Hand pump water	38(84.4)	24(53.3)	10.161	0.001
Filtered water	7(15.6)	21(46.7)		
Contact with soil				
Yes	35 (77.8)	26 (57.8)	4.121	0.042
No	10 (22.2)	19 (42.2)		
Swimming in unregulated water bodies				
Yes	23(51.1)	12(26.7)	5.657	0.017
No	22(48.9)	33(73.3)		
Travel history				
Yes	10(22.2)	6(13.6)	1.112	0.292
No	35(77.8)	38(86.4)		

Table 3: Distribution of *G. intestinalis* in Laghman in relation to Season (One sample Chi-square Test)

Season	*Observed N*	*Expected N*	*Residual*	X^2	*P-value*
Spring	7	11.3	- 4.3	10.387	0.016
Summer	15	11.3	3.8		
Fall	18	11.3	6.8		
Winter	5	11.3	- 6.3		

G. intestinalis and associated risk factors in Nangarhar

The distribution of positive cases across age and sex in Nangarhar, shown in Figure 2, reveals higher positivity rates among children aged 5 to 14. Females had higher positivity rates than males across most age groups, a disparity that remained significant according to chi-square analysis and after adjusting for age (P=0.002). Older age was associated with a lower likelihood of testing positive (P<0.001).

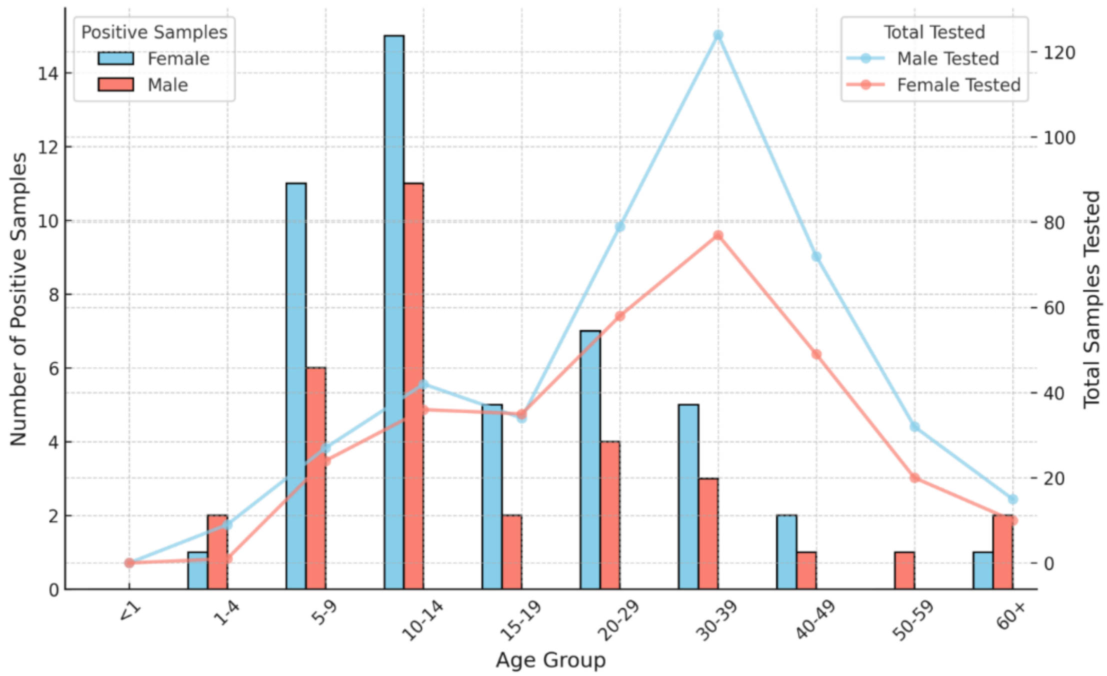

Figure 2: Distribution of positive cases across different age groups, stratified by sex, in Nangarhar. The number of positive samples is shown as bars (blue for females, red for males), while the total number of individuals tested per group is represented by lines. Positivity was higher among children aged 5–14 yr compared to other age groups. Females exhibited a significantly higher positivity rate compared to males, which remained significant after adjusting for age (*P*=0.002). Age influenced positivity, with older individuals being less likely to test positive (*P*<0.001).

Monthly income was significantly associated with infection (*P*=0.018), with the highest rates observed among participants from high-income families (
Table 4). Seasonal variation was also significant (*P*=0.045), with infections peaking in summer (Table 5).

Environmental and behavioral factors, such as domestic animal ownership, source of drinking water, soil contact, swimming in unregulated water bodies, and travel history, were not statistically significant. However, those who owned domestic animals or consumed unfiltered water showed higher positivity rates.

Comparative analysis of G. intestinalis risk factors in Laghman and Nangarhar

The comparative analysis of *G. intestinalis* risk factors between Laghman and Nangarhar revealed regional disparities in risk factors (Correlation between ***G. intestinalis infection and clinical symptoms***

As shown in Table 7, the most common symptoms among positive cases were abdominal pain (83.9%) and diarrhea (60.5%), with watery (51.6%) and bloody (9.7%) forms. Other frequent symptoms included fever (59.7%), nausea (58.9%), weakness (31.5%), vomiting (25%), and headache (24.2%).

Table 6). Sex distribution was similar in both provinces, but males in Laghman had significantly higher positivity rates than those in Nangarhar (*P*=0.002). Participants in Nangarhar reported significantly higher monthly incomes than those in Laghman (*P*<0.0001).

Environmental and behavioral factors also differed significantly. Swimming in unregulated water bodies ($P<0.0001$), domestic animal ownership ($P=0.001$), and soil contact ($P<0.0001$) were all more common in Laghman and significantly associated with *G. intestinalis* infection. In contrast, travel history ($P=0.536$) and source of drinking water ($P=0.12$) showed no significant association with positivity.

Table 4: Distribution of *G. intestinalis* in Nangarhar in relation to demographic, socioeconomic, environmental, and behavioral factors

Risk Factors	***Case (N = 79) n (%)***	***Controls (N = 79) n (%)***	X^2	***P-value***
Socioeconomic Factors				
Monthly income				
<10,000 AFN	12 (85.7)	2 (14.3)	7.991	0.018
10,000 – 20,000 AFN	30 (44.8)	37 (55.2)		
>20,000 AFN	37 (48.1)	40 (51.9)		
Environmental and Behavioral Factors				
Domestic Animals				
Yes	39 (54.2)	33 (45.8)	1.418	0.234
No	37 (44.6)	46 (55.4)		
Source of drinking Water				
Hand pump water	57 (51.8)	53 (48.2)	0.472	0.604
Filtered water	22 (45.8)	26 (54.2)		
Contact with soil				
Yes	34 (52.3)	31 (47.7)	0.235	0.628
No	45 (48.4)	48 (51.6)		
Swimming in unregulated water bodies				
Yes	14 (48.3)	15 (51.7)	0.042	1
No	65 (50.4)	64 (49.6)		
Travel history				
Yes	21 (46.7)	24 (53.3)	0.183	0.668
No	56 (50.5)	55 (49.5)		

Table 5: Distribution of *G. intestinalis* in Nangarhar in relation to Season (One sample Chi-square Test)

Seasons	***Observed Numbers***	***Expected Numbers***	***Residual***	χ^2	***P-value***
Spring	19	19.8	- 0.8	8.038	0.045
Summer	30	19.8	10.3		
Fall	17	19.8	- 2.8		
Winter	13	19.8	- 6.8		

Correlation between G. intestinalis infection and clinical symptoms

As shown in Table 7, the most common symptoms among positive cases were abdominal pain (83.9%) and diarrhea (60.5%), with watery (51.6%) and bloody (9.7%) forms. Other frequent symptoms included fever (59.7%), nausea (58.9%), weakness (31.5%), vomiting (25%), and headache (24.2%).

Table 6: Comparative Analysis of associated risk factors of G. intestinalis between Laghman and Nangarhar

Characteristics	***Laghman (n = 45), n (%)***	***Nangarhar (n = 79), n (%)***	***X^2***	***P-value***
Sex				
Male	31 (68.9)	32 (40.5)	9.24	0.002
Female	14 (31.1)	47 (59.5)		
Monthly income (AFN)				
< 10,000	50 (55.6)	12 (15.2)	41.504	
10,000 – 20,000	32 (35.6)	30 (38.0)		< 0.0001
> 20,000	8 (8.9)	37 (46.8)		
Swimming in unregulated water bodies				
Yes	23 (51.1)	14 (17.7)	15.267	< 0.0001
No	22 (48.9)	65 (62.3)		
Domestic animals				
Yes	36 (80.0)	39 (50.6)	10.331	0.001
No	9 (20.0)	38 (49.4)		
Source of drinking water				
Hand-pump Water	38 (84.4)	57 (72.2)	2.418	0.12
Filtered Water	7 (15.6)	22 (27.8)		
Contact with soil				
Yes	35 (77.8)	34 (43.0)	14.019	<0.0001
No	10 (22.2)	45 (57.0)		
Travel history				
Yes	10 (22.2)	21 (27.3)	0.382	0.536
No	35 (77.8)	56 (72.3)		
Season				
Spring	7 (15.6)	19 (24.1)	5.190	0.158
Summer	15 (33.3)	30 (38.0)		
Autumn	18 (40.0)	17 (21.5)		
Winter	5 (11.1)	13 (16.5)		

Table 7: Distribution of positive cases of *G. intestinalis* on the basis of clinical symptoms

Symptoms	***Laghman (n = 45) n (%)***	***Nangarhar (n = 79) n (%)***	***Total (N = 124) N (%)***
Abdominal pain			
Yes	37 (82.2)	67 (84.8)	104 (83.9)
No	8 (17.8)	12 (15.2)	20 (16.1)
Diarrhea			
Yes	35 (79.5)	40 (50.6)	75 (60.5)
No	9 (20.5)	39 (49.4)	48 (38.7)
Type of diarrhea			
Watery	26 (86.7)	38 (82.6)	64 (51.6)
Bloody	4 (13.3)	8 (17.4)	12 (9.7)
Fever			
Yes	25 (55.6)	49 (62.0)	74 (59.7)
No	20 (44.4)	30 (38.0)	50 (40.3)
Nausea			
Yes	24 (53.3)	49 (62.0)	73 (58.9)
No	21 (46.7)	30 (38.0)	51 (41.1)
Weakness			
Yes	14 (31.1)	25 (31.6)	39 (31.5)

No	31 (68.9)	54 (68.4)	85 (68.5)
Vomiting			
Yes	13 (28.9)	18 (22.8)	31 (25.0)
No	32 (71.1)	61 (77.2)	93 (75.0)
Headache			
Yes	10 (22.2)	20 (25.3)	30 (24.2)
No	35 (77.8)	59 (74.4)	94 (75.8)

Discussion

This study assessed *G. intestinalis* positivity and risk factors among individuals with gastrointestinal symptoms in Laghman and Nangarhar provinces, revealing a higher positivity rate in Nangarhar (10.6%) than in Laghman (7.5%). Notably, the positivity rate in Laghman was lower than in most provinces except Wardak, while Nangarhar exceeded those in Wardak, Khost, and Kabul (18, 20, 21).

Several significant risk factors for *G. intestinalis* infection were identified in Laghman, including male sex, domestic animal ownership, soil contact, swimming in unregulated water bodies, consumption of untreated or unfiltered water, and seasonal variations in positivity rates. The significantly higher positivity rates of *G. intestinalis* among males in Laghman align with findings from Khost and Pakistan (21, 31). The increased prevalence in males may be due to their more active role in society, particularly in outdoor work and agriculture, which expose them to higher infection risks. In Laghman, the primary livelihood is agriculture and farming, involving close contact with livestock, soil, and water – potential sources of *G. intestinalis* contamination. A study in Iraq supports this, linking the higher prevalence in males to their increased activity in public places and workplaces, as well as greater exposure to infection through eating and drinking at street vendors (32).

Domestic animal ownership (80%) and soil contact (77.8%) showed strong associations with increased transmission, likely due to zoonotic exposure and inadequate hygiene following soil contact. The zoonotic potential of *G. intestinalis* has been highlighted in several studies, especially in its transmission between animals and humans through direct contact or contaminated environments. For example, in India, *G. intestinalis* subtype AI was detected in both calves and farmers working on the same farm (33). Soil contact contributes to infection, specifically in areas where animal manure and human waste are used as fertilizers, facilitating zoonotic and anthroponotic transmission. This is consistent with the findings of Resi et al., who identified a significant link between gardening activities and elevated positivity rates (34). In Laghman, where agriculture is the primary livelihood, year-round exposure to livestock, soil, and water sources further heightens the risk of infection, as individuals frequently interact with potential sources of contamination.

Waterborne transmission of *G. intestinalis* is a significant concern, particularly in regions with inadequate water treatment and sanitation infrastructure. In most provinces of Afghanistan, including Laghman, there are no formal swimming pools. The primary swimming areas for locals are the unregulated natural water bodies, such as lakes, rivers, and agricultural reservoirs. In Northern Greece, 66.2% of surface water samples were contaminated with *G. intestinalis* (35). In the present study, individuals in Laghman exhibited a higher tendency to swim, thereby increasing their exposure to *G. intestinalis,* as water bodies are susceptible to contamination by parasites and their cysts. Contributing factors to this contamination may include poor drainage systems and runoff from fields fertilized with animal and human waste. A similar association was found between the prevalence of *G. intestinalis* and swimming in contaminated pools (36). This association has also been reported in other studies, particularly those documenting similar outbreaks in swimming pools (37-39). These observations underscore the importance of public health initiatives aimed at educating communities about safe swimming practices, as well as the need for the government to establish regulated swimming pools for the public and ensure their regular disinfection to mitigate the risks associated with waterborne transmission.

The consumption of untreated hand pump water was a significant risk factor in Laghman (*P*=0.001), consistent with studies linking higher positivity rates to limited access to safe drinking water (13, 17, 40). Insufficient access to clean water can increase the infection risk by up to 14% (6), emphasizing the critical need for improved water treatment and filtration systems in areas with limited access to potable water.

Seasonal variations were also significant in Laghman, with *G. intestinalis* infections peaking in autumn, followed by summer, spring, and winter (*P*=0.016). This autumn peak aligns with findings from Canada, where positivity rates also surged in early autumn (41). These seasonal trends are likely influenced by environmental factors such as temperature and rainfall, which impact the survival and transmission of the parasite.

In Nangarhar, sex, age, monthly income, and seasonal variations were significantly associated with *G. intestinalis*. Females exhibited a significantly higher positivity rate compared to males. This finding is consistent with a study in Ethiopia, which also reported a higher prevalence of intestinal parasitic infections, including *G. intestinalis*, among females, attributing it to their exposure through household tasks such as food preparation, cleaning, and collecting water (42). The significant association between age and *G. intestinalis* positivity in Nangarhar suggests that younger individuals may be at greater risk, likely due to higher exposure to contaminated sources. This aligns with the findings of other studies conducted both within Afghanistan and in other countries (18, 20, 43).

Interestingly, despite global trends showing higher infection rates in low-income groups, our study found greater prevalence among higher-income individuals in Nangarhar. The positivity rates in human populations range from 4% to 43% in low-income and 1% to 7% in high-income countries (44). Many studies have reported a high prevalence in low-income populations (45). However, our findings revealed a higher prevalence among higher-income individuals in Nangarhar. Although limited data exists on risk factors for high-income populations, insights from Krumrie et al.'s recent study identify contaminated water and contact with diaper-wearing children as the two most common risk factors in higher-income communities (46). Many individuals in Nangarhar are involved in trade and businesses, which may expose them to the endemic regions of Afghanistan and Pakistan along the Torkham route. As a result, their families may also be at risk, potentially contributing to the higher prevalence in this population. The involvement of chefs and food handlers from low-income families further facilitates disease transmission. A study by AL-Mekhlafi et al. found a prevalence of 4.4% among food handlers in Yemen, while Aber et al. found a higher prevalence of 7.0% among food handlers in Ethiopia. Both studies suggested an association between untrained and unhygienic food handlers and food-borne transmission of *G. intestinalis* (47, 48). Other studies also support the anthroponotic transmission of specific *G. intestinalis* assemblages, particularly among adults involved in diaper changing and cleaning children in the daycare center (49).

Seasonal variations of positivity rates in Nangarhar revealed higher positivity rates in the summer, followed by spring, autumn, and winter (*P*=0.045). This pattern is consistent with findings from other studies conducted within the country, including those in Wardak and Kandahar provinces (17, 20), as well as in other countries such as the US, Canada, and the UK, where giardiasis exhibited a more pronounced peak during the summer (50, 51).

The comparative analysis between the two provinces revealed that several risk factors were consistent with those identified in individual provincial analyses. Notably, the positivity rate was significantly higher among males in Laghman compared to Nangarhar. In contrast, although females in Nangarhar exhibited higher positivity rates, this difference was not statistically significant. These findings are consistent with previous research. For instance, Khudhair reported sex-based differences in prevalence across three cities in Northern Iraq, with higher prevalence among males in Hawler and females in Chamchamal and Soran (52). Several factors may contribute to the observed differences between males and females. Differences in exposure to contaminated water, occupational roles, or hygiene practices may cause these sex disparities. Further investigation is needed to explore the underlying causes of these sex-specific differences in *G. intestinalis* transmission.

Other significant risk factors included soil contact, swimming in unregulated water bodies, and domestic animal ownership, reinforcing their importance in the epidemiology of *G. intestinalis* in Laghman. In Nangarhar, a high monthly income was identified as a significant risk factor in the separate and comparative analyses. This consistent finding suggests a potential link between socioeconomic status and the positivity rate of *G. intestinalis* in Nangarhar, indicating the need for further studies to explore this association.

Although seasonal variations were significant in each province, with autumn peaks in Laghman and summer peaks in Nangarhar, no significant difference was observed in the comparative analysis of the two provinces. This may be due to prolonged warm climates in both provinces.

Children aged 5 to 14 exhibited the highest rates of *G. intestinalis* infection in both provinces (Figures 1 and 2). This pattern reflects a combination of environmental exposure and infrastructural limitations specific to the region. In rural Afghanistan, children in this age group often participate in farming and animal husbandry, which increases their contact with contaminated soil, untreated water sources, and animal waste. The lack of safe recreational alternatives means children swim in polluted rivers and irrigation canals, further elevating their exposure. Many schools lack functional toilets, access to clean drinking water, and proper handwashing facilities, all of which significantly contribute to the risk of transmission. Additionally, overcrowded classrooms with poor conditions create a setting conducive to the spread of infection. These interrelated factors likely explain the heightened burden of *G. intestinalis* among school-aged children in these communities.

In this study, using a single stool sample per patient likely led to an underdiagnosis of *G. intestinalis* infections, given the known limitations of this method. The sensitivity of microscopy using a single sample is approximately 46%, while examining three samples over 3–5 d increases accuracy to 94% (53). However, even this approach has proven to be less sensitive than ELISA. For instance, in a study involving Polish soldiers in Ghazni, Afghanistan, microscopy detected a positivity rate of 2.7%, whereas ELISA identified a significantly higher rate of 18.1% (19). Similarly, among children in Pakistan, microscopy found a prevalence of 2.75%, while ELISA detected 9.5% (40). Despite these differences, microscopic examination of three stool samples per patient remains the gold standard for routine diagnosis, while ELISA and other more sensitive methods are recommended in cases where clinical symptoms persist despite negative microscopy results (54).

Hygiene and sanitation also play a critical role in preventing *G. intestinalis* infection, as highlighted by a comparison of soldiers deployed in Afghanistan. German soldiers adhering to strict food and water hygiene protocols exhibited a positivity rate of 1.3% (55), whereas Polish soldiers without comparable sanitary measures showed a significantly higher positivity rate of 18.1% (19). This contrast underscores the importance of sanitation and access to clean water in controlling the transmission.

Conclusion

This study detected *G. intestinalis* in 7.5% of symptomatic individuals in Laghman and 10.6% in Nangarhar, indicating a notable burden among those with gastrointestinal complaints. Sex was a significant factor in both provinces, while age was associated with infection only in Nangarhar, suggesting regional epidemiological differences. Since the sample included only symptomatic individuals, the findings may underestimate community prevalence, particularly of asymptomatic cases. The lack of data on co-infections limits understanding of the parasite's specific clinical impact. Future research should employ community-based sampling, utilize sensitive diagnostics such as ELISA or PCR, and evaluate co-infections to better estimate the prevalence and disease burden.

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Conflict of interest

The authors declare that there is no conflict of interests.

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