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Clinical, Environmental and Anthrozoönotic Factors Associated with Giardiasis in a Kenyan Rural Setting

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Abstract

Background: Giardiasis is an important neglected tropical disease caused by the enteric protozoal parasite *Giardia intestinalis*. The infection is often asymptomatic but cases of symptomatic infections lead to significant morbidity and mortality especially in the developing nations. However, the transmission of this infectious disease largely involves complex interplay of demographic, environmental and zoonotic factors attracting medical and veterinary approaches in delineating its epidemiology. To date, only a few studies have been conducted in Kenya focusing prevalence, less is known about the factors associated with giardiasis in a rural setting. As such, this hospital-based cross-sectional case-control study evaluated the demographic, clinical, environmental and anthrozoönotic factors associated with giardiasis in a rural area of Western, Kenya. A total of 147 individuals referred to the laboratory for stool analysis; cases (n = 78) and controls (n = 69), were recruited into the study. Demographic analysis indicated, gender, age < 18 year, underweight, overweight, ≤ primary education levels, small-scale business, subsistence farming, peri-urban residences, mud-walled, grass-thatched roof, unscreened window houses, household with < 3 members, history of travel or eating at hotels, funeral and from food vendors, were associated with likelihood of giardiasis ($P < 0.05$). Likewise presenting with fever, headache, vomiting, bloating, stomach upset, abdominal pain, diarrhoea and history of nitroimidazole use were associated with giardiasis ($P < 0.05$). Stool analysis showed; semi-formed, loose, pus cells, mucus, foul smelling or greasy stools were associated with giardiasis. Sources of water; boreholes, shallow well, stream, spring or pipetted, chlorination as well as storage in super-drums had higher odds for giardiasis, while boiling of drinking water and handwashing had lower odds for giardiasis ($P < 0.05$). Having cattle, goats, poultry, and/or cats at home were also associated with higher odds for giardiasis ($P < 0.05$). Altogether, these results indicated that demographic, environmental and zoonotic factors are important factors in the transmission of giardiasis in this rural setting.

Keywords: Giardiasis, *Giardia intestinalis*, clinical, environmental and anthrozoönotic factors, rural setting

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Introduction

Giardiasis is a major neglected contagious disease of humans, domestic and wild animals caused by the protozoan parasite *Giardia intestinalis* (also known as *G. lamblia* or *G. duodenalis*) (1). Nearly, 183 million people in the world are infected with *G. intestinalis*, and the African region experiencing 809 cases per 100,000 population each year (2,3). Additionally, giardiasis contributes about 171 thousands disability life adjusted years (DALYs), globally with almost 50% occurring in the African region (2,4). Furthermore, giardiasis is a common enteric disease accounting for nearly 20% of water-borne protozoan disease outbreaks in the world from 2017 to 2020 (5). In Kenya, the correct prevalence of giardiasis is unknown. However, based on available studies, an estimated *G. intestinalis* prevalence of 5-45% has been reported in two rural areas (6,7). In Busia county, the current study setting, 33% of out-patients complaining of abdominal symptoms had microscopically confirmed giardiasis (8). However, the underlying factors contributing to the high burden of giardiasis in rural settings are not fully understood.

The consumption of treated water, hand washing with soap and clean water before eating and after visiting the toilet, residing in an informal settlement, and undernutrition are associated with giardiasis in urban settings (9,10). Likewise, gender, younger age, weight, underweight and overweight, less than primary school level education, working on the farm, living in earthen floor or grass thatched houses, history of travel and history of eating at hotels, cafeteria and from food vendors are linked to *G. intestinalis* infection in rural Africa (9,11,12). Additionally, ingestion of cyst contaminated water, such as from boreholes, shallow wells, streams, rivers, springs, and poor treatment and storage of water are correlated with increased risk of *G. intestinalis* infection in rural areas (10). Furthermore, studies in Uganda, Ethiopia and Malaysia have indicated that interactions with domestic or wild animals, such as cattle, dogs, cats and wild primates is connected to a greater risk of getting giardiasis (13–15). From the aforementioned, it appears that socio-demographic, environmental and zoonotic

factors, including water safety are important factors in the epidemiology of giardiasis in rural Africa.

Giardiasis results from ingestion of the infective cysts in contaminated food or water, as well as from direct faeco-oral or anal sexual contact (9,16). Subsequently, the parasite migrates and infects the duodenum and ileum causing local and systemic inflammation. From the inflammation, cytokine release and other soluble mediators trigger fever, headache and gastrointestinal symptoms that characterize the disease. Furthermore, upon entering the enterocytes the parasite cause damage and loss of epithelial brush border which is accompanied with microvilli shortening and loss of function (17). Even though, some patients remain asymptomatic and void formed stool, destruction of the intestinal wall with exfoliation of epithelial cells leads to a variable degree of passing foul smelling, semi-formed or loose stools containing pus cells, mucus or steatorrhea in symptomatic patients (18,19). Additionally, the history of nitroimidazole (metronidazole, albendazole, secnidazole, or tinidazole) use can alter the course and severity of the clinical manifestations of the *G. intestinalis* infections (20,21). Although, the constitutional symptoms and signs of the disease are not pathognomonic, microscopic confirmation of the presence of the giardia trophozoite and/or cysts in the stool specimens is the gold standard widely used in guiding treatment and preventive programs. As such, this study aimed at examining the relationship of demographic, clinical, environmental and zoonotic interaction with *G. intestinalis* infection in a rural setting of Western Kenya.

Material and Methods

Study area, study design and population. This hospital-based case-control study was conducted at Busia County Referral Hospital, Busia County, Western Kenya. Busia County is located to the North of Lake Victoria bordering Uganda and currently is experiencing an influx of people in search of better economic opportunities. It has a bimodal weather pattern comprising of a wet season from April to October and a dry season from November to March. The main economic

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activities in the county include small-scale businesses, subsistence farming, including sugarcane farming, livestock rearing, fishing and keeping of pet animals such as dogs and cats (22). The county has a high population density of 527 people per square kilometer, an average household size of 5 people, literacy levels of 56% and a poverty index of 66% (23,24). The study comprised 147 patients presenting with abdominal complaints and referred to the clinical laboratory for stool examination. The cases and controls were defined by the presence or absence of microscopically confirmed *G. intestinalis* trophozoites and/or cysts, respectively.

Inclusion and exclusion criteria. Only consenting individuals with abdominal complaints and referred to the clinical laboratory for stool analysis were enrolled into the study. Individuals declining consent, and those unable to provide a stool specimen at the time of visiting the hospital were excluded from the study.

Information and stool specimen collection. A structured questionnaire was used to collect demographic information (gender, age, height, weight, education level, occupation, house floor, window and roof type, household size, history of eating at hotel, funeral or from food vendors, residence (either rural or peri-urban) and travel history (outside the county or country). Height was measured to the nearest 0.1 cm using a Holtain stadiometer (Holtain Ltd, UK), while the body weight was measured to the nearest 0.1 kg using a portable digital electronic balance (AND Fv-150 KA1, A&D Ltd, Japan). The body mass index (BMI, kg/m^2) was calculated from the weight and height measurements and classified into normal ($\geq 18.5 < 25.0$), underweight (< 18.5), overweight (≥ 25.0) based on the WHO classification of the nutrition status (25). A checklist questionnaire was used to record the patients' presenting history of signs and symptoms (fever or temperature $> 37^\circ\text{C}$,

headache, vomiting, bloating, stomach upset, abdominal pain and diarrhoea experienced in the preceding two weeks before visiting the hospital for clinical care. Besides, history of nitroimidazole use (metronidazole, ornidazole or secnidazole), tinidazole, and/or albendazole use within the previous two weeks prior to the study was also recorded. Environmental information on water sources (boreholes, shallow wells, streams, river, spring, rain water and piped water), treatment (boiling or chlorination), and storage (jerricans, tank or super drum), domestic and pet animals (cattle, goat, sheep and poultry, dogs and cats) were recorded.

Stool sampling and analyses was performed as per the Center for Diseases Control and Prevention (CDC) guidelines on laboratory identification of parasites of medical importance in stool specimens (26). Briefly, upon receipt of stool examination request form from the doctor, each patient, patient's parent or guardian was provided with a clean and leak-proof plastic container with an applicator stick attached to a well fitted screw cup. Each patient was then requested to aseptically collect a stool sample into the stool container. The stool specimen was immediately transported to the laboratory for macroscopic (formed, semi-formed, loose, pus cells, mucus, foul smell or greasy stool) and microscopic (saline and iodine wet preparation) analysis. A pigeon pea size (~1.0 g) stool sample was placed on a dry clean microscope slide, emulsified using a drop of 0.9% normal saline or 5% Lugol's iodine and covered with a clean 22-mm cover slip. The microscope slide was then examined under objective lens x10 and x40 for the presence and quantification of trophozoites and cysts of *G. intestinalis*, respectively (Figure 1). The parasite density (trophozoite or cyst/ μL of stool) was classified as scanty (1-3), few (4-10), moderate (11-20), many (21-40) and very many (> 40) trophozoites and/or cysts (27).

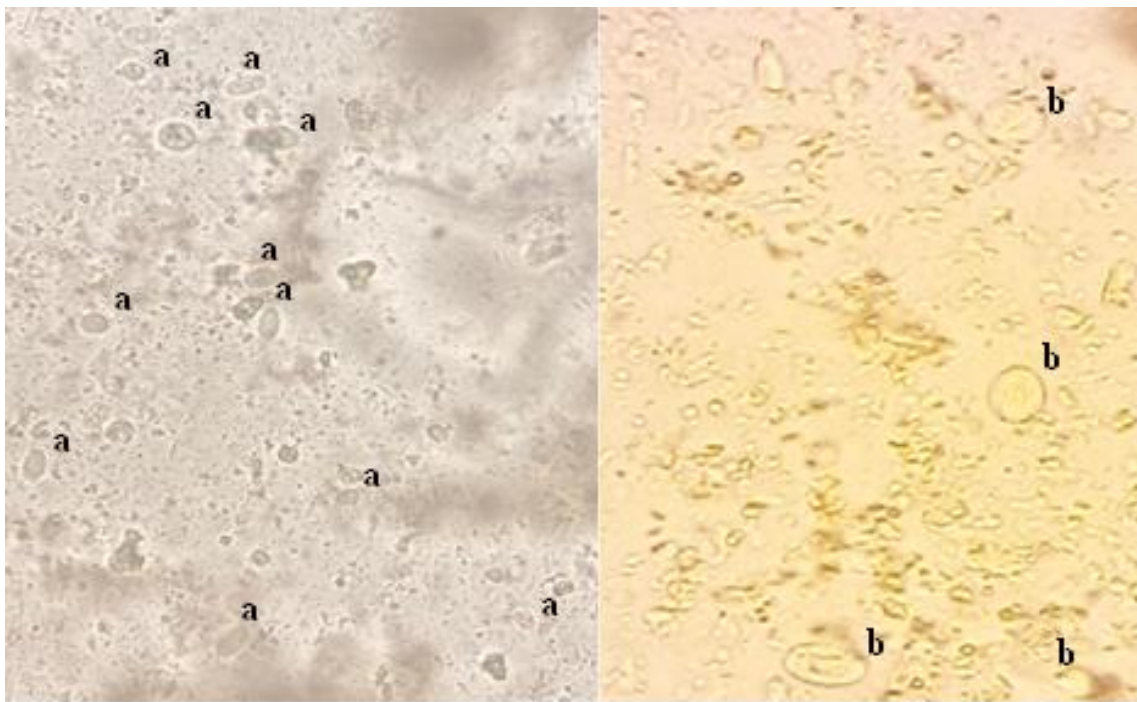


Figure 1. Giardia intestinalis trophozoites (a) and cysts (b) on 0.9% normal saline wet mount and 5% Lugol's iodine wet preparation at x40 objectives.

Data management and analysis. Data was analyzed using the statistical package for the social sciences (IBM® SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp., USA). Categorical variables (gender, nutrition status, occupation, clinical, environmental and anthrozoonotic factors) were compared between the cases and controls using the Fisher's exact tests or the Pearson's Chi-square test. Continuous variables (age, weight, height, and BMI) were compared between the cases and controls using the Mann Whitney U tests. Associations of the demographic, clinical, environmental and anthrozoonotic factors with giardiasis was determined using the binary logistic regression analyses. All tests were two-tailed and a value of $P < 0.05$ was used for statistical inferences.

Ethical consideration. This study was conducted in accordance with the tenets of the Helsinki Declaration. Ethical approvals were sought from the Maseno University Ethical Review Committee (Protocol: MU/403012-V36). The research permit was obtained from the Busia County Referral Hospital Management. A written consent was obtained from each adult and parent/guardian of each child prior to enrollment into the study. The consent form clearly indicated the study purpose,

any anticipated consequences of the research, the anticipated uses of the data, possible benefits and harm, data confidentiality, and the option to withdraw from participation. The positive cases of giardiasis participants received treatment as per the Ministry of Health (MoH) in Kenya guidelines.

Results

Demographic characteristics of the study patients. The demographic characteristics of the study participants are presented in Table 1. There were more males among the cases (cases, 62.8% vs. controls, 40.6%; OR, 2.474; 95% CI, 1.273 - 4.809; $P = 0.008$) with higher odds for giardiasis. Although, age was similar between the cases and controls, a majority of the cases were younger (<18) years and had higher probability of giardiasis (cases, 53.8% vs. controls, 20.3%; OR, 4.194; 95% CI, 2.194 - 9.573; $P < 0.0001$). Assessment of the physical measures showed lower body weights in the cases (median [IQR] kg; cases, 57.0 [41.0] vs. controls, 70.0 [27.0]; $P = 0.038$). Similarly, the cases had lower BMI (median [IQR] kg/m²; cases, 19.0 [10.2] vs. controls, 25.0, [8.0]; $P = 0.021$), and higher rates of underweight (cases, 18.9% vs. controls, 16.2%; OR, 2.784; 95% CI, 1.312 - 5.907; $P = 0.008$) and overweight (cases, 44.6% vs. controls 26.5%; OR,

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2.362; 95% CI, 1.897 - 6.219; $P = 0.022$) that were linked to higher odds of presenting with giardiasis.

Evaluation of the education profiles showed that most of the patients had primary education which was associated with *G. intestinalis* infection (cases, 69.2% vs. controls, 44.9%; OR, 2.314; 95% CI, 1.133 - 4.726; $P = 0.027$). Besides, a majority of the patients engaged in small-scale businesses (cases, 57.7% vs. control, 42.0%; OR, 2.211; 95% CI, 1.001 - 4.882; $P = 0.004$), and subsistence farming (cases, 17.9% vs. controls, 5.8%; OR, 1.534; 95% CI, 1.381 - 3.239; $P = 0.003$) which had higher odds of giardiasis. Similarly, most of the cases resided in peri-urban areas, and had higher odds of giardiasis (cases, 71.8% vs. controls, 46.4%; OR, 2.201; 95% CI, 1.141- 4.360; $P = 0.024$). Assessment of the housing structure indicated that most of the cases residing in the mud-walled houses were likely to have *G. intestinalis* infection (cases, 34.6% vs. controls, 18.8%; OR, 2.938; 95% CI, 1.501 -

5.753; $P = 0.002$). Likewise, patients residing in grass-thatched houses (cases, 16.7% vs. controls, 7.2%; OR, 5.743; 95% CI, 1.821 - 8.119; $P = 0.003$) or houses with unscreened windows (cases, 54.8% vs. controls, 18.6%; OR, 3.278; 95% CI, 1.122 - 4.635; $P = 0.003$) dominated among the cases and had higher probability of having giardiasis. In addition, most households with greater than 3 people were more prevalent in the cases and were associated with higher risk of giardiasis (cases, 53.2% vs. controls, 23.2%; OR, 2.911; 95% CI, 1.376 - 6.158; $P = 0.005$). Evaluation of the history of travel and eating habits indicated that most individuals in the cases had a history of travel (cases, 42.3% vs. controls, 21.7%; OR, 2.640; 95% CI, 1.276 - 5.464; $P = 0.009$), eating at hotels (cases, 85.9% vs. controls, 56.5%; OR, 1.692; 95% CI, 1.718 - 3.985; $P = 0.026$), eating at funerals (cases, 23.1% vs. 7.2%; OR, 3.560; 95% CI, 1.863 - 7.596; $P = 0.046$) or eating from food vendors (cases, 20.5% vs. 8.7%; OR, 2.710; 95% CI, 1.995 - 7.378; $P = 0.031$) which were all associated with greater probability of presenting with giardiasis.

Table 1: Demographic profiles of the study patients

Characteristics	Cases, n=78	Controls, n=69	P	OR	95% CI	*P	
Gender, n (%)							
Male	49 (62.8)	28 (40.6)	0.007	2.474	1.273-4.809	0.008	
Female	29 (37.2)	41 (59.4)		Ref			
Age, years	24.3 (24.9)	25.3 (24.5)	0.315				
<18	42 (53.8)	14 (20.3)	<0.0001	4.194	2.194-9.573	<0.0001	
≥18	36 (46.2)	55 (79.7)		Ref			
Height, cm	156.0 (39.0)	158.0 (15.0)	0.473	-	-	-	
Weight, kg	57.0 (41.0)	70.0 (27.0)	0.038	-	-	-	
BMI, kg/m ²	19.0 (10.2)	25.0 (8.0)	0.021	-	-	-	
Nutrition status, n (%)							
Underweight	14 (18.9)	9 (16.2)	0.017	2.362	1.897-6.219	0.022	
Overweight	33 (44.6)	18 (26.5)		2.784	1.312-5.907		0.008
Normal	27 (36.5)	18 (26.5)		Ref			
Education, n (%)							
≤Primary	54 (69.2)	31 (44.9)	0.025	2.314	1.133-4.726	0.027	
≥Secondary	24 (30.8)	38 (55.1)		Ref			
Occupation, n (%)							
Small-scale business	45 (57.7)	29 (42.0)	<0.0001	2.211	1.001-4.882	0.004	
Subsistence farming	14 (17.9)	4 (5.8)		1.534	1.381-3.239		0.003
Formal employment	19 (24.4)	36 (52.2)		Ref			
Residence, n (%)							

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Peri-urban	56 (71.8)	32 (46.4)	0.023	2.201	1.141-4.360	0.024
Rural	22 (28.2)	27 (53.6)		Ref		
<i>House type, n (%)</i>						
Mud-walled	27 (34.6)	13 (18.8)	<0.0001	2.938	1.501-5.753	0.002
Brick-walled	51 (65.4)	56 (81.2)		Ref		
<i>Roof type, n (%)</i>						
Grass-thatched	13 (16.7)	5 (7.2)	0.001	5.743	1.821-8.119	0.003
Tin roofed	65 (83.4)	64 (92.8)		Ref		
<i>Window type, n (%)</i>						
Unscreened	34 (54.8)	11 (18.6)	0.002	3.278	1.122-4.635	0.003
Screened	28 (45.2)	48 (81.4)		Ref		
<i>Household size, n (%)</i>						
>3	33 (53.2)	16 (23.2)	0.006	2.911	1.376-6.158	0.005
1-3	29 (46.8)	53 (76.8)		Ref		
<i>Travel history, n (%)</i>						
Yes	33 (42.3)	15 (21.7)	0.019	2.640	1.276-5.464	0.009
No	45 (57.7)	53 (76.8)		Ref		
<i>Eating history, n (%)</i>						
Hotels						
Yes	67 (85.9)	39 (56.5)	0.021	1.692	1.718-3.985	0.026
No	11 (14.1)	30 (43.5)		Ref		
Funerals						
Yes	18 (23.1)	5 (7.2)	0.043	3.560	1.863-7.596	0.046
No	60 (76.9)	63 (91.8)		Ref		
Food vendors						
Yes	16 (20.5)	6 (8.7)	0.030	2.710	1.995-7.378	0.031
No	62 (79.5)	62 (89.9)		Ref		

Data presented are number (n) and proportion (%) of subjects and as odds ratios (OR) with 95% confidence interval (CI). BMI; body mass index; Normal; $\geq 18.5 \leq 25.0 \text{ kg/m}^2$, Underweight $< 18.5 \text{ kg/m}^2$, Overweight $\geq 25.0 \text{ kg/m}^2$. ^PFisher's exact tests; ^RChi-square test, *P*; **P*, logistic regression analysis. Bolded are significant *P*-values.

Presenting history. The presenting history of the patients is summarized in Table 2. Assessment of the patient presenting history indicated greater rates of fever (cases, 50.0% vs. controls, 31.9%; OR, 2.136; 95% CI, 1.090 - 4.189; *P* = 0.027), headache (cases, 51.3% vs. controls, 27.5%; OR, 2.770; 95% CI, 1.389 - 5.523; *P* = 0.004), vomiting (cases, 44.9% vs. controls, 27.5%; OR, 2.142; 95% CI, 1.073 - 4.277; *P* = 0.031), bloating (cases, 65.4% vs. controls, 17.4%; OR, 8.972; 95% CI, 4.122-19.531; *P* < 0.0001), stomach

upset (cases, 47.4% vs. controls, 29.0%; OR, 2.211; 95% CI, 1.116-4.382; *P* = 0.023), abdominal pain (cases, 62.8% vs. controls, 44.9%; OR, 2.071; 95% CI, 1.070 - 4.008; *P* = 0.038), diarrhoea (cases, 52.6% vs. controls, 13.0%; OR, 4.546; 95% CI, 3.222 - 16.936; *P* < 0.0001) except history of nitroimidazole use (cases, 37.2% vs. controls, 53.6%; OR, 0.326; 95% CI, 0.010-0.777; *P* = 0.034) and were associated with increased risk of presenting with giardiasis.

Table 2. Presenting history of the study participants

	Cases, n=78	Controls, n=69	<i>P</i>	OR	95% CI	* <i>P</i>
<i>Signs and symptoms</i>						
Fever, n (%)						
Yes	39 (50.0)	22 (31.9)	0.026	2.136	1.090-4.189	0.027

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No	39 (50.0)	47 (68.1)		Ref		
Headache, n (%)						
Yes	40 (51.3)	19 (27.5)	0.003	2.770	1.389-5.523	0.004
No	38 (48.7)	50 (72.5)		Ref		
Vomiting, n (%)						
Yes	35 (44.9)	19 (27.5)	0.030	2.142	1.073-4.277	0.031
No	43 (55.1)	50 (72.5)		Ref		
Flatulence, n (%)						
Yes	18 (23.1)	17 (24.6)	0.825	0.918	0.429-1.962	0.827
No	60 (76.9)	52 (75.4)		Ref		
Bloating, n (%)						
Yes	51 (65.4)	12 (17.4)	<0.0001	8.972	4.122-19.531	<0.0001
No	27 (34.6)	57 (82.6)		Ref		
Stomach upset, n (%)						
Yes	37 (47.4)	20 (29.0)	0.022	2.211	1.116-4.382	0.023
No	41 (52.6)	49 (71.0)		Ref		
Abdominal pain, n (%)						
Yes	49 (62.8)	31 (44.9)	0.031	2.071	1.070-4.008	0.038
No	29 (37.2)	38 (55.1)		Ref		
Diarrhoea, n (%)						
Yes	41 (52.6)	9 (13.0)	<0.0001	4.546	3.222-16.936	<0.0001
No	37 (47.4)	60 (87.0)		Ref		
Nitroimidazole use, n (%)						
Yes	29 (37.2)	37 (53.6)	0.030	0.326	0.010-0.777	0.034
No	49 (62.8)	32 (46.4)		Ref		

Data presented are number (n) and proportion (%) of subjects and as odds ratios (OR) with 95% confidence interval (CI). ^PFisher's exact tests; ^RChi-square test, *P*; **P*, logistic regression analysis. Bolded are significant *P*-values.

Stool analysis. A summary of the stool characteristics is presented in Table 3. Stool examination revealed that voiding formed (cases, 2.6% vs. controls, 60.9%; OR, 0.005; 95% CI, 0.004 - 0.008; *P* < 0.0001), semi-formed (cases, 51.3% vs. controls, 30.4%; OR, 2.406; 95% CI, 1.221 - 4.741; *P* = 0.0011) or loose (cases, 61.5% vs. controls, 43.5%; OR, 2.080; 95% CI, 1.076 - 4.021; *P* = 0.029) stools, and presence of pus cells (cases, 37.2% vs. controls, 8.7%; OR, 6.214; 95% CI, 2.391 - 16.149; *P* < 0.0001), mucus (cases, 55.1% vs. controls, 7.2%; OR, 8.726; 95% CI,

5.707-15.333 - 16.149; *P* < 0.0001), foul smell (cases, 50.0% vs. controls 13.0%; OR, 6.667; 95% CI, 2.909 - 15.279; *P* < 0.0001) and steatorrhea (cases, 53.8% vs. controls, 13.0%; OR, 7.778; 95% CI, 3.3391 - 17.838; *P* < 0.0001) in the faeces were associated with giardiasis. Furthermore, enumeration of the trophozoite densities indicated a median (IQR) level of 7.0 (4.0) trophozoites/μl of stool with most of the patients having few (60.5%) or moderate (13.2%) parasite densities.

Table 3. Stool characteristics of the *G. intestinalis* infected individuals

Stool characteristics	Cases, n=78	Controls, n=69	<i>P</i>	OR	95% CI	* <i>P</i>
Formed, n (%)						
Yes	2 (2.6)	42 (60.9)	<0.0001	0.005	0.004-0.008	<0.0001
No	76	27 (39.1)		Ref		
Semi-formed, n (%)						

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Yes	40	21 (30.4)	0.010	2.406	1.221-4.741	0.011
No	38	48 (69.6)		Ref		
Loose, n (%)						
Yes	48	30 (43.5)	0.029	2.080	1.076-4.021	0.029
No	30	39 (56.5)		Ref		
Pus cells, n (%)						
Yes	29	6 (8.7)	<0.0001	6.214	2.391-	<0.0001
No	49	63 (91.3)		Ref		
Mucus, n (%)						
Yes	43	5 (7.2)	<0.0001	8.726	5.707-	<0.0001
No	35	64 (92.8)		Ref		
Foul smell, n (%)						
Yes	39	9 (13.0)	<0.0001	6.667	2.909-	<0.0001
No	39	60 (87.0)		Ref		
Steatorrhea, n (%)						
Yes	42	9 (13.0)	<0.0001	7.778	3.3391-	<0.0001
No	36	60 (87.0)		Ref		
Trophozoites/ μ l stool, median	7.0 (4.0)	-				
<i>Trophozoites levels, n (%)</i>						
Scanty	20	-				
Few	46	-				
Moderate	10	-				

Data presented are number (n) and proportion (%) of subjects and as odds ratios (OR) with 95% confidence interval (CI). Parasite density were expressed as numbers (n) and proportion (%) of scanty (1-3) trophozoites, few (4-10) trophozoites and moderate (11-20) trophozoites. ^PFisher's exact tests; ^RChi-square test, *P*; **P*, logistic regression analysis. Bolded are significant *P*-values.

Water sources, treatment and storage practices.

A summary of water sources, treatment, storage and hygiene practices is shown in Table 4. Evaluation of water sources showed that boreholes (cases, 66.7% vs. controls, 31.9%; OR, 2.273; 95% CI, 2.140 - 8.530; *P* < 0.0001), shallow wells (cases, 26.9% vs. controls, 8.7%; OR, 3.510; 95% CI, 1.596 - 9.354; *P* = 0.003), and streams (cases, 71.8%) vs. controls, 47.8%; OR, 2.777; 95% CI, 1.403 - 5.497; *P* = 0.004) were the major sources of water and had higher odds of presenting with *G. intestinalis* infection. Boiling (cases, 7.7% vs. controls, 21.7%; OR, 0.300; 95% CI, 0.109 - 0.804; *P* = 0.020) and

chlorination (cases, 21.8% vs. controls, 8.7%; OR, 0.926; 95% CI, 0.082 - 7.915; *P* = 0.034) were the main water treatment methods associated with giardiasis. Among storage methods reported, use of super drums dominated in the cases (cases, 78.1% vs. controls, 59.4%; OR, 2.451; 95% CI, 1.192 - 5.039; *P* = 0.015) and was associated with increased risk of giardiasis. Furthermore, hand washing before meals (cases, 38.5% vs. controls, 76.8%; OR, 0.821; 95% CI, 0.071 - 0.915; *P* = 0.020) and after visiting the toilet (cases, 69.2% vs. controls, 88.4%; OR, 0.721; 95% CI, 0.182 - 0.827; *P* = 0.036) was linked to lower risk of *G. intestinalis* infection.

Table 4. Water sources, treatment, storage and hygiene practices

Practices	Cases, n=78	Controls, n=69	<i>P</i>	OR	95% CI	* <i>P</i>
<i>Water sources, n (%)</i>						
Borehole						
Yes	52 (66.7)	22 (31.9)	<0.0001	2.273	2.140-8.530	<0.0001

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No	26 (33.3)	47 (68.1)		Ref		
Shallow well						
Yes	21 (26.9)	6 (8.7)	0.002	3.510	1.596-9.354	0.003
No	57 (73.0)	62 (91.3)		Ref		
Stream						
Yes	56 (71.8)	33 (47.8)	0.003	2.777	1.403-5.497	0.004
No	22 (28.2)	36 (52.2)		Ref		
River						
Yes	16 (20.5)	15 (21.7)	0.856	0.929	0.420-2.054	0.856
No	62 (79.5)	54 (78.3)		Ref		
Spring						
Yes	6 (7.7)	0 (0.0)	0.018	-	-	-
No	72 (92.3)	69 (100.0)		-	-	-
Rain water						
Yes	69 (88.5)	60 (87.0)	0.781	1.150	0.429-3.084	0.783
No	9 (11.5)	9 (13.0)		Ref		
Piped water						
Yes	13 (16.7)	0 (0.0)	<0.0001	-	-	-
No	65 (83.3)	69 (100.0)		-	-	-
<i>Water Treatment, n (%)</i>						
Boiling						
Yes	6 (7.7)	15 (21.7)	0.015	0.300	0.109-0.804	0.020
No	72 (92.3)	54 (78.3)		Ref		
Chlorination						
Yes	17 (21.8)	6 (8.7)	0.029	2.926	1.082-7.915	0.034
No	61 (78.2)	63 (91.3)		Ref		
<i>Water Storage, n (%)</i>						
Jerricans						
Yes	75 (96.2)	68 (98.6)	0.623	2.720	0.276-6775	0.691
No	3 (3.8)	1 (1.4)		Ref		
Super drums						
Yes	61 (78.1)	41 (59.4)	0.014	2.451	1.192-5.039	0.015
No	17 (21.8)	28 (40.6)		Ref		
Tanks						
Yes	9 (11.5)	13 (18.8)	0.216	1.178	0.709-4.466	0.219
No	69 (88.5)	56 (81.2)		Ref		
<i>Hygiene practices, n (%)</i>						
Hand washing before meal						
Yes	30 (38.5)	53 (76.8)	0.017	0.821	0.071-0.915	0.020
No	48 (61.5)	16 (23.2)		Ref		
Hand washing after toilet						
Yes	54 (69.2)	61 (88.4)	0.035	0.721	0.182-0.827	0.036
No	24 (30.8)	8 (11.6)		Ref		

Data presented are number (n) and proportion (%) of subjects and odds ratios (OR) with 95% confidence interval (CI). ^PFisher's exact tests; ^RChi-square test, *P*; **P*, logistic regression analysis. Bolded are significant *P*-values.

Association of domestic and common pet animals with giardiasis. A summary of common domestic and pet animals associated with

giardiasis is presented in Table 5. Analysis of the relationship of commonly kept domestic and pet animals with giardiasis illustrated that cattle

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(cases, 43.6% vs. controls, 30.4%; OR, 1.623; 95% CI, 1.554 - 6.052; $P = 0.042$), goats (cases, 21.8% vs. controls, 1.4%; OR, 8.951; 95% CI, 2.449 - 6.648; $P = 0.005$), poultry (cases, 48.7% vs. controls, 33.3%; OR, 1.574; 95% CI, 1.297 -

7.110; $P = 0.046$), and cats (cases, 39.7% vs. controls, 11.6%; OR, 5.029; 95% CI, 2.117 - 8.949; $P < 0.0001$) were prevalent in the cases and were linked to greater chances of having giardiasis.

Table 5. Domestic and pet animals

Domestic animals and pets, n (%)	Cases, n=78	Controls, n=69	P	OR	95% CI	* P
Cattle						
Yes	34 (43.6)	21 (30.4)	0.036	1.623	1.554-6.052	0.042
No	44 (56.4)	48 (69.6)		Ref		
Goat						
Yes	17 (21.8)	1 (1.4)	<0.0001	8.951	2.449-6.648	0.005
No	61 (78.2)	68 (98.6)		Ref		
Poultry						
Yes	38 (48.7)	23 (33.3)	0.034	1.574	1.297-7.110	0.036
No	40 (51.3)	46 (66.7)		Ref		
Dog						
Yes	24 (30.8)	17 (24.6)	0.408	1.359	0.656-2.817	0.409
No	54 (69.2)	52 (75.4)		Ref		
Cat						
Yes	31 (39.7)	8 (11.6)	<0.0001	5.029	2.117-8.949	<0.0001
No	47 (60.3)	61 (88.4)		Ref		

Data presented are number (n) and proportion (%) of subjects and as odds ratios (OR) with 95% confidence interval (CI). ^PFisher's exact tests; ^RChi-square test, P ; * P , logistic regression analysis. Bolded are significant P -values.

Discussion

Demographic characteristic of the study patients.

Demographic evaluations indicating that more males and younger people (age <18 years) were likely to have giardiasis, suggests that the male gender and children are more likely to interact with potential risks of *G. intestinalis* infection. This is probably related to poor hygienic practices such as handwashing after toilet visit and before eating. Additionally, adventurous lifestyles such as hunting, gardening, hiking and grazing can lead to increased exposure with potential risks of *G. intestinalis* infections (28). Furthermore, the higher rates of physical activities among younger people, and the fact that children frequently play with soil and surface waters which are often contaminated with giardia parasites predisposes them to acquiring the disease. Consistent with this findings, a cross-sectional school-based study in South West Ethiopia indicated that male children were two times at risk of intestinal parasitic

infections (29). In addition, school-based correlation study in North East of Ethiopia showed that male students had higher odds of having intestinal parasitic infections (30). On the contrary, previous studies in Western Kenya in an area with similar environmental conditions as the present study showed that females had higher rates of giardiasis (8). Furthermore, the study findings are consistent with epidemiological studies on intestinal parasites in the community and in informal settlements in Kenya indicating higher rates of infection among children (31,32). Interestingly, breast-fed children had a low prevalence of giardiasis (33,34), suggesting a protective value of breast milk during early childhood.

The low body weight and body mass index of the giardiasis patients, suggests that *G. intestinalis* infection triggers gastrointestinal inflammation leading to malabsorption, maladigestion, loss of nutrients such as ADEK vitamins, and

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competition for nutrients especially micronutrients (copper, zinc and iron) with the host resulting in malnutrition (35). In agreement with these results, a number of studies showed higher rates of weight loss, underweight and muscle wasting among children presenting with giardiasis (36–39). In addition, increased levels of protein energy malnutrition was associated with giardiasis in children (40,41). However, the finding of higher prevalence of overweight individuals among giardiasis patients can be ascribed to the malabsorption and constipation state in the patients. On this premise, *G. intestinalis* infection can modulate metabolism via changing the gut microbiome composition, promoting local intestinal inflammation, and altering appetite, food intake and the body mass index (42,43). Subsequently, these changes can cause fat deposition over time leading to obesity (44). These assertions are further supported by results of a survey in a Mexican population showing that an increase in the body mass index of 0.6 kg/m² was associated with intestinal parasitic infections (45).

The results of having more individuals with primary or less education, more individuals engaging in small scale business and subsistence farming in the cases, suggests that poor health education practices among this predominantly subsistence population is a critical factor in increasing the risk of *G. intestinalis* infections. Consistent with the findings, school-based cross-sectional studies in two primary schools in Northeast Ethiopia reported high prevalence of parasitic infections among children with uneducated parents (30). In addition, children of uneducated or primary level educated mothers had greater risk of presenting with giardiasis in Ghana (46), Tanzania (47) and Cuba (48). This is further supported by results indicating that individuals with formal employment were about two times less likely to have giardiasis. This means that possessing at least secondary education and being in stable formal employment are associated with appropriate health practices such as good health seeking behavior, access to health finances and good hygiene practices. This proposition is supported by results among school-going children in informal settlements of Nakuru town, Kenya indicating that having a parent practicing business was linked to a higher

probability of intestinal infections (49). Likewise, studies among children hospitalized with diarrhea in Goiânia, Brazil, revealed that children who lived on a farm were likely to get infected with *G. intestinalis* (13). Equally important are observations that sugarcane chewing is connected to high infection rates among primary school children in North East Ethiopia (49,50). On the contrary, studies among school-going children in informal settlements in Nakuru, Kenya showing that children from families practicing farming were less likely to be infected with intestinal parasites or geohelminths (49), suggest that farming is not a risk factor of intestinal parasitic infections.

A peri-urban residence was associated with higher odds of having *G. intestinalis*, suggesting that peri-urban settings have higher sources of giardiasis. The possible underlying reasons include poor water quality, inappropriate sewage disposal and sanitation facilities, unhygienic eateries and high population densities which promote contamination and transmission of giardia cysts. Consistent with this results, previous studies in Ethiopia indicated high prevalence of intestinal parasites among urban residents relative to inhabitants from rural areas (30). Similarly, higher rates of giardiasis were reported in the informal settlements of urban and peri-urban regions in Kenya (32,49). These hypotheses are further supported by the results of the present study showing that fewer rural residents had *G. intestinalis*.

Dwelling in mud-walled or grass-thatched houses, houses with unscreened windows or in households with more than three people was associated with higher rates of giardiasis, suggesting increased indoor contamination leading to infection. Mud-walled crevices harbor giardiasis transmission agents like small rodents, lizards and cockroaches (48,49,50), and the practice of geophagy in which pregnant women eat soil from the mud walls (9). Given that soil harbors giardia cyst for longer periods, it represents a critical source of infection in pregnant women craving for soil, and children contaminating their hands and objects that they ingest (9,54). Besides, unscreened windows let contaminated dust in the dry season to enter the house and contaminate food and water resulting in infection (6). Additionally, this findings, partly

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concur with a study in Nakuru, Kenya reporting higher prevalence of intestinal parasites among children from families with few rooms (49). Similarly, case-control studies in England and Brazil showed that children from households with more than four children had higher odds of having giardiasis (55,56).

A history of travel and eating at hotels, funerals or from food vendors was associated with higher likelihood of giardiasis, suggesting increased ingestion of foods and water contaminated with the infectious giardia cysts. Consistent with the current study, a history of travel to endemic regions was associated with giardiasis in the developed countries (28,57,58). For instance, a prospective cohort study in the Netherland among long-term (3-12 months) travelers to Sub-Saharan Africa, reported high incidence of *G. intestinalis* infection (12). Similarly, individuals with a history of travelling in England were three times at risk of giardiasis (55). Furthermore, a history of eating from outdoor food outlets is an important predictor of intestinal infections. This observation is consistent with previous studies among children from an informal settlement in Kenya indicating that eating from school was associated with the likelihood of intestinal protozoal infection (49). Likewise, studies among pregnant women in Ghana showed that intestinal parasitic infections were more prevalent in women consuming cooked food from vendors (54).

Presenting history. The presenting complains of fever, headache and gastrointestinal symptoms were associated with giardiasis, indicating that *G. intestinalis* presents with both gastro-intestinal and systemic manifestations. The gastrointestinal symptoms are due to duodenal and ileal giardia infection releasing parasite metabolic products and toxins that cause local inflammation. In addition, endogenous mediators with pyrogenic effect such as interleukin (IL)-1 β , IL-6 and tumor necrosis factor- α secreted from leukocytes and other cells during giardia infection stimulate fever and headache (59). Furthermore, similar symptoms have been reported in children presenting with giardiasis in Rwanda (60), Ethiopia (61) and Egypt (19). On the contrary, genetic variability of the *G. intestinalis* parasite is associated with differences in the clinical presentation of the disease. For instance, studies

in the Southwest of London, England among giardiasis cases showed that both assemblages A and B caused similar illnesses, but assemblage A was more frequently associated with fever than assemblage B (62). Similarly, among children less than 5 years of age in Spain, sub-assemblage AII was associated with symptomatic infection, while assemblage B was correlated with asymptomatic infection (63). Thus, genetic diversity influences the virulence and clinical outcomes of *G. intestinalis* infection.

Stool analysis. Stool examination results showing that passing stools that are semi-formed, loose or that are containing pus cells, mucus, greasy or foul smelling is associated with the likelihood of *G. intestinalis* infection, suggests that local parasite induced inflammation underlies the mechanism of gastrointestinal manifestations. Consistent with this results, previous studies reported that voiding semi-formed or loose stools, presence of mucus, steatorrhea and foul smelling stool were frequent features of symptomatic giardiasis (64,65). However, assemblage A *G. intestinalis* infection was associated with steatorrhea in children from Canada, suggesting that stool characteristics depends on parasite virulence (66). The basic mechanisms leading to semi-formed, loose or diarrheagenic stools is due to enterocyte damage and loss of brush border of the epithelial cells, consequently shortening the microvilli and modulating epithelial barrier functions (17). Besides, steatorrhea and mucus release result from decreased activity of lipases and increased release of mucin from goblet cells (67,68). Additionally, the presence of pus cells and foul smelling stools is attributable to intestinal epithelial cell apoptosis (4). The results of a median number of trophozoites of 7.0 with higher rates of scanty and few levels of trophozoites, suggest a more acute or recent infection. This results are similar to previous studies reporting scanty and few levels of trophozoites in children with acute giardiasis in western Kenya (69,70). On the contrary, previous studies indicating scanty and few levels of trophozoites in patients with history of nitroimidazole use, suggest refractory disease due to treatment failure (71).

Water sources, treatment storage and hygienic practices. Results showing that the major water sources (boreholes, shallow well, stream, spring

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and pipetted water) are associated with *G. intestinalis* infection, suggest presence of the infective giardia cysts. However, boiling is an effective method of water treatment compared to chlorination. In addition, storage using super drums appears to be a risky means of water storage. Altogether, these results suggest higher rates of water contamination especially at sources and during storage. The possible reason includes contamination via collecting vessels, dust and soil particles, surface run-off water and farm animal excreta. Consistent with these results, giardia parasites have been detected in well water in Mexico (72). Similarly, a high prevalence of giardiasis was observed in children from Nakuru Kenya, using borehole water sources (49). Although piped water sources seem to be associated with lower odds of giardiasis in the current study, some studies showed that these water sources can also be contaminated (56,73). With regard to water treatment, boiling was associated with reduced odds, whereas chlorination was correlated with the likelihood of giardiasis. This is attributable to the use of sub-optimal chlorine dose, pH and temperature rendering chlorination ineffective. Additionally, contamination of water treatment plants with cysts from technical complications of coagulation, filtration, disinfection or broken pipes may result in *G. intestinalis* infection. These results also correspond with the CDC guidelines stipulating that boiling is the most effective method of drinking water treatment, while chemical chlorination is ineffective against giardia cysts (74). Storage of water in super drums is ineffective in sustaining safety over a long period as it may allow dust particles and other contaminants to enter open drums. Likewise, stored water can be contaminated through use of dirty collecting vessels, whereas rainfall water can be contaminated by washing of dust particles on the roof into the collecting reservoirs. Consistent with these observations, rain water and spring water have been associated with intestinal parasitic infections, which was attributed to contamination of the storage facilities (18).

The hygienic practices comprising hand washing before meals and after visiting the toilet were associated with low possibility of giardiasis,

suggesting a reduction in the load of viable infective cysts of *G. intestinalis*. However, a subset of individuals who practiced hand washing also had giardiasis, and this is probably due to poor hand washing practices like not using soap and safe water during hand washing. In agreement with these results, a lack of or poor hand washing techniques have been associated with transmission of intestinal parasites among children inmates (75–77). On the contrary, a randomized trial in Kenya among children who practiced handwashing as a strategy to control giardiasis, was ineffective in reducing the infection (76), suggesting that other risk factors are involved either directly or indirectly in the transmission of giardiasis.

Association of domesticated and common pet animals with giardiasis. Giardiasis seems to have a zoonotic link in this rural community as evidenced by the higher rates of *G. intestinalis* infections in individuals residing in homes keeping cattle, goats, poultry and cats. This could be attributed to the fact that a majority of people interact with cattle dung, goat pellets and poultry excreta plus their pet companions that predisposes them to infection. In addition, a number of households co-inhabit with animals in their dwellings, especially earthen kitchens with weaning calves, kids and poultry, thus increasing the risk of contamination. Furthermore, use of cattle and goat excreta as manure, eating of raw fruits and vegetable like sweet potatoes and carrots, and the habit of chewing sugarcane without proper washing are potential sources of infections in this rural area. Although, cats are regarded as clean animals, it is possible that they can carry the giardia cysts in their furs, particularly during the dry season, and transmit the parasite during interaction with their owners. Consistent with these results, epidemiological studies in Belgium (78), Canada, (79,80) and informal settlements in Kenya (81) have reported increased risk of zoonotic transmission of giardiasis. Furthermore, previous studies in Kisumu, Kenya, detected high *G. intestinalis* presence in stool samples of animals roaming the city (82). However, studies in Nakuru, Kenya among school-going children failed to show any association of goat rearing with the likelihood of intestinal infections (49). Additionally, previous studies among children in Brazil, implicated cats

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in the transmission of giardiasis (50), while in the Pacific Region, cats were linked to increased odds of *G. intestinalis* infection in children (83). Moreover, having cat pets was associated with intestinal protozoan parasites among school children from informal settlements in Nakuru, Kenya (49).

Conclusion

The results of this study indicated that demographic (age, gender, education, occupation, residence, house type, household size, history of travel and history of eating (at a funeral, hotel, or food vendor in the preceding two weeks), gastrointestinal complaints, stool examination, environmental (domestic water sources, water treatment, water storage, hygienic practices, and anthroponotic (domestic animals and pets) factors are important in defining clinical presentation and acquisition of giardiasis. This study has implications on the epidemiology, as well as control and prevention of the disease in resources limited rural settings.

Limitations of the Study

Although the current cross-sectional study provides important information on the factors defining giardiasis in a rural setting, a longer prospective study design will provide additional insights into the dynamics of *G. intestinalis* transmission. Even though information on travel and history of eating was obtained by recording recall in the preceding fortnight, generalization of the results is valid as most of the patients had at least high school education and could discern the details about their prior health history. The use of microscopic stool examination for defining *G. intestinalis* positive cases is appropriate as this is the “gold-standard” clinical stool analysis, including examination of the stool specimens on three occasions by experienced microscopists. However, molecular identification can be a complementary investigation to provide additional information about the isolates, assemblages and sub-assemblages.

Data Availability

The data used to support the findings of the current study are available from the corresponding author upon request.

Competing Interest

None of the authors have a commercial relationship or financial conflict of interest as part of this study.

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Authors' Contributions

TW and RI conceived and designed the study. RI, NS and EB performed the experiments. EB and TW performed statistical analyses, interpreted the results, and co-drafted and revised the manuscript together with NS. All authors have read and approved the manuscript.

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