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The triple threat of *Cryptosporidium*, *Giardia*, and *Entamoeba* infections in Nigerian children

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The epidemiological patterns and risk factors of three protozoan parasites, *Cryptosporidium*, *Giardia*, and *Entamoeba*, that cause diarrheal diseases in low-resource settings were investigated among children aged 10 years and below across ten states in Nigeria. Previous studies in Nigeria relied primarily on microscopy, a method with limited sensitivity for parasite detection, which underestimated the true burden of infection. We applied a real-time PCR technique on DNA extracted from 977 stool samples collected from children who answered a structured questionnaire in this cross-sectional survey. The overall prevalence of *Cryptosporidium*, *Giardia*, and *Entamoeba* infections was 18.1%, 77.6%, and 12.3%, respectively. Coinfections were frequent, with 17.1% harbouring both *Cryptosporidium* and *Giardia*, 2.9% with *Cryptosporidium* and *Entamoeba*, 11.5% with *Giardia* and *Entamoeba*, and 2.8% with all three parasites. Jigawa State recorded the highest burden of coinfections (7.9%). *Cryptosporidum* infection rates were significantly higher in children five years and above, while males had higher *Entamoeba* infection rates. *Giardia* infection varied significantly with educational level. Trading was significantly associated with the burden of single infections and *Cryptosporidium* and *Giardia*, and *Giardia* and *Entamoeba* coinfections. Risk factors for infection and coinfection included geographical location, water source, household density, and contact with animals. The study highlights the importance of integrating molecular diagnostic tools into public health surveillance to accurately assess parasitic disease burdens and guide interventions. The high prevalence of *Giardia*, in particular, underscores the urgent need to improve access to clean water, sanitation, and hygiene (WASH) infrastructure in Nigeria. Addressing these infections through improved diagnostics, sanitation infrastructure, and targeted interventions could substantially reduce childhood morbidity and mortality associated with diarrheal diseases.

Keywords Children, *Cryptosporidium*, *Entamoeba*, *Giardia*, Nigeria, qPCR

Abbreviations

VIF	Variance inflation factor
WHO	World Health Organization
qPCR	Quantitative PCR

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WASH	Water, sanitation, and hygiene
FMOH	Federal Ministry of Health

Cryptosporidium, *Giardia*, and *Entamoeba* are three common intestinal protozoa that cause diarrheal diseases in humans[1–3]. These parasites are significant contributors to morbidity and mortality, particularly in developing countries. *Cryptosporidium* and *Giardia* have been included in the World Health Organization (WHO) Neglected Diseases Initiative since 2004[4]. Children are particularly vulnerable to these infections due to their less developed immune systems and factors such as malnutrition[5]. The impact of *Cryptosporidium*, *Giardia* and *Entamoeba* infections on children's overall health can be particularly severe. These parasites, whether individually or in coinfection, can lead to malnutrition, anaemia, chronic severe diarrhoea, bowel obstruction, stunted growth, and impaired cognitive function, with severe cases potentially resulting in fatalities[5,6].

Cryptosporidium is the second leading cause of severe diarrhoea, accounting for approximately 20% of all cases of childhood diarrhoea in developing countries[7,8]. Malnourished young children and severely immunocompromised individuals are most at risk of cryptosporidiosis[9]. Giardiasis, caused by *Giardia intestinalis*, is the most prevalent enteric protozoan infection globally[2,10]. The tropical region bears roughly three-quarters of the global burden, with prevalence rates ranging from 0.1 to 60% in sub-Saharan Africa, and an estimated 8.97% in West Africa[11,12]. Higher risk groups include infants, young children, the elderly, immunocompromised individuals, people with diabetes or cystic fibrosis, and travellers[2,12]. *Entamoeba* is also a leading cause of diarrhoea in children under two years of age living in developing countries[13]. *Entamoeba* infections are endemic in tropical and subtropical low- and middle-income countries, particularly in areas with poor sanitation. Young children, especially those under five years, are most susceptible to gastrointestinal amoebiasis, while amoebic liver abscesses are more commonly seen in adult males[3].

The transmission of these protozoa occurs primarily via the faecal–oral route[1–3]. Infection can also occur through human-to-human contact or zoonotic transmission[1–3,14]. These parasites have simple life cycles without intermediate hosts. Cysts or oocysts (in the case of *Cryptosporidium*) are excreted in faeces, and infection typically results from ingestion of these infective stages[15].

Several risk factors have been linked to the transmission of these parasites, including inadequate sanitation, unsafe drinking water, and poor hygiene[1–3,14]. These socioeconomic challenges are widespread in low- and middle-income countries, including Nigeria. Consequently, the prevalence of these protozoan infections is significantly higher in such regions, posing a major public health challenge. Understanding their epidemiology is therefore critical for effective control and prevention.

Traditionally, stool microscopy has been considered the gold standard for diagnosing these infections. However, recent molecular diagnostic methods, such as quantitative PCR (qPCR), offer higher sensitivity and specificity[16]. Despite their advantages, molecular techniques are not yet widely implemented in routine diagnostic settings[16]. In areas like Nigeria, where microscopy remains the primary diagnostic tool, the burden of these parasites is likely underestimated due to diagnostic limitations[17]. To address this gap, the present study aimed to determine the molecular epidemiology and associated risk factors associated with *Cryptosporidium*, *Giardia*, and *Entamoeba* infections among children in Nigeria.

Methods

Ethic statement

Ethical approval was obtained from the ethics committees of the State Ministries of Health and Hospital Management Boards. Informed consent was obtained from the parents or caregivers of all participants. All methods were performed in accordance with relevant institutional guidelines and regulations, and in compliance with the Declaration of Helsinki. Details of the approving ethics committees are provided in the ethics statement section.

Sample design

A cross-sectional survey was conducted between August 2022 and February 2023 in ten states in Nigeria. The sampling strategy was based on a previous epidemiological study that assessed the prevalence of pathogenic protozoan infections in a similar population[18]. A total of 985 participants were recruited that comprised children less than 10 years of age from public primary schools' hospital outpatient departments or primary health care centers in ten states of Nigeria (Fig. 1). Inclusion criteria included the willingness of parents/guardians to provide informed consent, and the willingness of participants to provide fresh stool samples, of which 977 participants met these criteria. Exclusion criteria included a lack of informed consent, and severely sick individuals.

Sample collection

Participants were given sample bottles labelled with unique IDs to provide fresh stool samples. A questionnaire was administered to each participant to collect demographic and socioeconomic data. Freshly collected stool samples were mixed with an equal volume of 95% ethanol within an hour to preserve DNA integrity. The samples were then immediately transferred to the Molecular Parasitology laboratory at the National Institute of Allergy and Infectious Diseases, NIH, USA for molecular analysis.

DNA isolation

Owing to the large number of samples, DNA was isolated using the QIAsymphony SP equipment, which is intended for automated nucleic acid purification in molecular diagnostic applications. The Qiagen QIAsymphony PowerFecal Pro DNA Kit was used to pretreat the samples prior to loading them on the QIAsymphony following the manufacturer's instructions. After pretreatment, the supernatant was transferred to sterile 2 ml microtubes

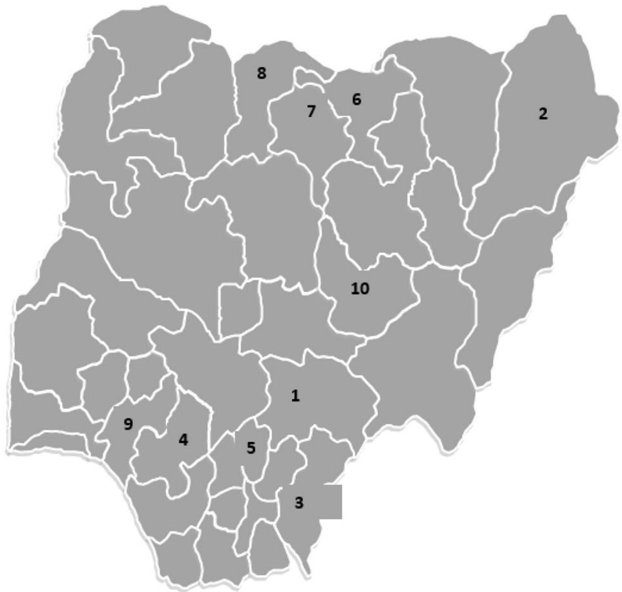

Fig. 1. Map of Nigeria showing study areas (States): 1. Benue 2. Borno 3. Cross River 4. Edo 5. Enugu 6. Jigawa 7. Kano 8. Katsina 9. Ondo 10. Plateau.

and placed on the QIAsymphony instrument for the bind DNA, washing and elution steps. The eluate was collected in a 96-well plate with the elution volume set at 100µL.

Real-time PCR detection

Using real time PCR amplification, the SSU rRNA gene fragment for *Cryptosporidium*, *Giardia*, and *Entamoeba* was amplified using the following GEMS primers[20] that were modified respectively, Crypto18SDF 5′-GGTTGTATTTATTAGATAAAGAAC-3′ and Crypto18SDR 5′-GTAGGCCAATACCCTACCGTCTAA-3′ (127bp); Giardia-80F 5′-GACGGCTCAGGACAACGGTT-3′ and Giardia-127R—5′-TTGCCAGCGGTGTCCG-3′ (62bp); Ehd-239F 5′-ATTGTCGTGGCATCCTAACTCA-3′ and Ehd-88R 5′-GCGGACGGCTCATTATAACA-3′ (172bp)[16]. For each experiment, 2 µl of template DNA was added to qPCR assays comprising 5µL SYBR green, 0.1pM of each primer in a final volume of 10 µL. Each experiment was performed in duplicates and appropriate controls (negative and positive controls) were included. The amplification parameters were as follows, 10 min at 95 °C followed by 40 cycles of 10 s at 95 °C and 40 s at 60 °C using the Quant 6 real-time PCR instrument (Applied Biosystems). Samples were considered positive for the parasite if the mean CT (threshold cycle) values were < 40 (Supplementary Tables 1–3).

Data analysis

The questionnaire data were entered into Microsoft Excel (Version 2412) and then imported into R software (Version 4.4.2) for summary statistics based on the sociodemographic characteristics of the study population. The chi-square test was used to examine the association between infection status and sociodemographic variables. Logistic regression analysis was performed to identify risk factors for single and coinfections with *Cryptosporidium*, *Giardia*, and *Entamoeba*. Infection status was treated as a binary outcome variable. Predictor variables were included in the regression model after assessing multicollinearity and influential observations using Variance Inflation Factor (VIF) and Cook's Distance, respectively. Some levels within categorical variables were removed prior to regression analysis due to lack of observations which led to overfitting and convergence problems in R. These adjustments were made to improve model stability and interpretability. Pearson correlation was used to assess the strength and direction of associations between continuous variables. The significance level for all statistical tests was set at 0.05 .

Results

The socio-demographic and clinical characteristics of the 977 participants with complete data are summarized in Table 1. The participants comprised children aged 10 years and below, with a mean age of 2.7 years. Among the participants, 483 were female and 494 were male, representing children from 10 different states. A significant proportion of the heads of households had attained tertiary education (32.5%), while trading was the predominant occupation, accounting for 40.1%. In terms of socioeconomic status, the majority of households (70.4%) were classified as having low socioeconomic status. Notably, 65.5% of the children did not exhibit symptoms of diarrhoea.

The overall prevalence of *Cryptosporidium* was 18.1%, with the highest burden observed in Enugu State. *Giardia* was detected in 77.6% of participants, with the highest prevalence recorded in Edo State. *Entamoeba* affected 12.3% of the population, with the highest prevalence in Jigawa State (Fig. 2, Table 2).

Variable	N	%
Age (Years)		
5–10	215	22.0
< 5	762	78.0
Mean (SD)	2.7 (3.2)	
Gender		
Female	483	49.4
Male	494	50.6
States		
Benue	94	9.6
Borno	79	8.1
Cross River	97	9.9
Edo	70	7.2
Enugu	99	10.1
Jigawa	89	9.1
Kano	196	20.1
Katsina	98	10.0
Ondo	68	7.0
Plateau	87	8.9
Head of household education level		
Informal	183	18.7
Primary	213	21.8
Secondary	264	27.0
Tertiary	317	32.5
Occupation		
Artisanal	129	13.2
Civil service	212	21.7
Farming	197	20.2
Self employment	19	1.9
Skilled worker	28	2.9
Trading	392	40.1
Socioeconomic status		
Low	688	70.4
Medium	289	29.6
Symptomology		
Asymptomatic	640	65.5
Symptomatic	337	34.5

Table 1. Sociodemographic and clinical characteristics of the study population.

Coinfections were also common: *Cryptosporidium* + *Giardia* was observed in 17.1% of the study population, *Cryptosporidium* + *Entamoeba* in 2.9%, *Giardia* + *Entamoeba* in 11.5%, and 2.8% of participants were coinfected with all three species. *Cryptosporidium* + *Giardia* coinfection was most prevalent in Enugu State, while *Cryptosporidium* + *Entamoeba*, *Giardia* + *Entamoeba*, and *Cryptosporidium* + *Giardia* + *Entamoeba* were most common in Jigawa State (Fig. 2, Table 3).

Children aged five years and older had significantly higher frequencies of *Cryptosporidium* infection (P = 0.04) (Tables 2 and 3). In terms of gender, males exhibited significantly higher infection rates for *Entamoeba* infections (P = 0.03) (Tables 2 and 3). Higher educational level was significantly associated with *Giardia* infection prevalence (P = 0.01). Among the different occupational categories, traders had the highest level of infection and was significantly associated with the burden of various infections, except for *Cryptosporidium* + *Entamoeba* and triple coinfections (P = 0.20 and P = 0.17) (Tables 2 and 3). Additionally, a significantly higher prevalence was observed among children from low socioeconomic backgrounds for *Entamoeba* infections (P = 0.04) (Tables 2 and 3).

The multivariate logistic regression analysis for *Cryptosporidium, Giardia*, and *Entamoeba* single infections identified several significant risk factors, including gender (for *Entamoeba* only), state (for all three parasites), occupation (for *Entamoeba* only), water source (for all three parasites), type of toilet (for *Cryptosporidium* only), washing of vegetables (for *Cryptosporidium* and *Entamoeba* only), living with animals (for *Cryptosporidium* and *Giardia* only), and other infections (Table 4).

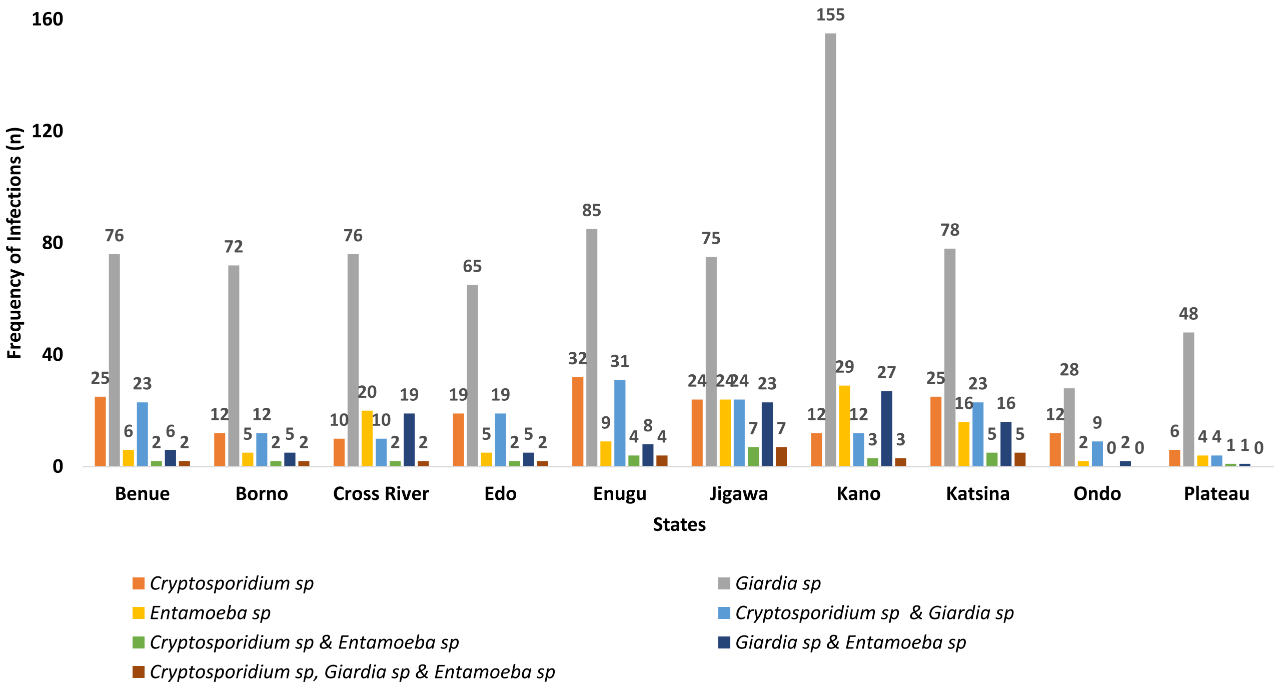

Fig. 2. Burden of *Cryptosporidium*, *Giardia* and *Entamoeba* infections according to locations.

Variable	Infecting species					
	Cryptosporidium		*Giardia*		*Entamoeba*	
	n (%)	P	n (%)	P	n (%)	P
Total[a]	177 (18.1)		758 (77.6)		120 (12.3)	
Age (years)						
5–10	128 (72.3%)	**0.04**	593 (78.2%)	0.74	100 (83.3%)	0.13
< 5	49 (27.7%)		165 (21.8%)		20 (16.7%)	
Gender						
Female	94 (53.1%)	0.28	378 (49.9%)	0.62	48 (40.0%)	**0.03**
Male	83 (46.9%)		380 (50.1%)		72 (60.0%)	
Edu. level						
Informal	38 (21.4%)	0.46	145 (19.1%)	**0.01**	25 (20.8%)	0.16
Primary	41 (23.2%)		182 (24.0%)		26 (21.7%)	
Secondary	49 (27.7%)		198 (26.1%)		40 (33.3%)	
Tertiary	49 (27.7%)		233 (30.8%)		29 (24.2%)	
Occupation						
Artisanal	16 (9.0%)	**0.00**	91 (12.0%)	**0.03**	21 (17.5%)	**0.04**
C. Service	35 (19.8%)		156 (20.6%)		13 (10.8%)	
Farming	36 (20.3%)		159 (21.0%)		26 (21.7%)	
S. Employed	9 (5.1%)		16 (2.1%)		1 (0.8%)	
S. Worker	1(0.6%)		18 (2.3%)		4 (3.4%)	
Trading	80 (45.2%)		318 (42.0%)		55 (45.8%)	
SES						
Low	132 (74.6%)	0.18	544 (71.7%)	0.09	94 (78.3%)	**0.04**
Med	45 (25.4%)		214 (28.2%)		26 (21.6%)	

Table 2. Prevalence of *Cryptosporidium, Giardia* and *Entamoeba* infections according to sociodemographic characteristics. [a]N = 977. Bolded values are significant at P = 0.05. *Edu. Level* Head of household educational level, *C. Service* Civil service, *S. Employed* Self employed, *S. Worker* Skilled worker.

Variable	Coinfecting species							
	Cryptosporidium + Giardia		*Cryptosporidium + Entamoeba*		*Giardia + Entamoeba*		*Cryptosporidium + Giardia + Entamoeba*	
	n (%)	P	n (%)	P	n (%)	P	n (%)	P
Total[a]	167 (17.1)		28 (2.9)		112 (11.5)		27 (2.8)	
Age (years)								
5–10	122 (73.1%)	0.09	20 (71.4%)	0.40	94 (83.9%)	0.11	19 (70.4%)	0.33
< 5	45 (26.9%)		8 (28.6%)		18 (16.1%)		8 (29.6%)	
Gender								
Female	89 (53.3%)	0.27	13 (46.4%)	0.75	46 (41.1%)	0.06	12 (44.4%)	0.60
Male	78 (46.7%)		15 (53.6%)		66 (58.9%)		15 (55.6%)	
Edu. level								
Informal	37 (22.2%)	0.48	7 (25.0%)	0.66	21 (18.8%)	0.17	7 (25.9%)	0.72
Primary	38 (22.8%)		5 (17.9%)		26 (23.2%)		5 (18.5%)	
Secondary	45 (26.9%)		9 (32.1%)		38 (33.9%)		8 (29.7%)	
Tertiary	47 (28.1%)		7 (25.0%)		27 (24.1%)		7 (25.9%)	
Occupation								
Artisanal	13 (7.8%)	**0.00**	4 (14.3%)	0.20	19 (17.0%)	**0.05**	3 (11.1%)	0.17
C/Service	34 (20.4%)		2 (7.1%)		12 (10.7%)		2 (7.4%)	
Farming	35 (20.9%)		5 (17.9%)		24 (21.4%)		5 (18.5%)	
S. Employed	8 (4.8%)		0 (0.0%)		1 (0.9%)		0 (0.0%)	
S. Worker	1 (0.6%)		0 (0.0%)		3 (2.7%)		0 (0.0%)	
Trading	76 (45.5%)		17 (60.7%)		53 (47.3%)		17 (63.0%)	
SES								
Low	124 (74.3%)	0.23	22 (78.5%)	0.34	88 (78.6%)		21 (77.8%)	0.40
Med	43 (25.7%)		6 (21.4%)		24 (21.4%)		6 (22.2%)	

Table 3. Prevalence of *Cryptosporidium, Giardia* and *Entamoeba* coinfections according to sociodemographic characteristics. [a]N = 977, Bolded values are significant at P = 0.05. *Edu. Level* Head of household educational level, *C. Service* Civil service, *S. Employed* Self employed, *S. Worker* Skilled worker.

For *Cryptosporidium* infection, children residing in Kano or Plateau States were significantly less likely to be infected, with adjusted odds ratios indicating an 86% and 78% reduction in likelihood, compared to those in Borno State (OR = 0.14, P = 0.00, CI = 0.04–0.45; OR = 0.22, P = 0.02, CI = 0.06–0.80). Drinking well water was significantly associated with an increased likelihood of infection (OR = 1.78, P = 0.04, CI = 1.02–3.14) compared to those using borehole water. The use of pit latrines was associated with a significantly lower likelihood of *Cryptosporidium* infection compared to water closets (OR = 0.54, P = 0.02, CI = 0.32–0.90). Additionally, washing vegetables and rearing domestic fowl were significantly linked to higher odds of *Cryptosporidium* infection (OR = 1.67, P = 0.04, CI = 1.03–2.72; OR = 2.35, P = 0.01, CI = 1.27–4.37). Coinfection with *Giardia* was also a significant risk factor, with individuals infected with *Giardia* being 5.87 times more likely to be infected with Cryptosporidium (Table 4).

For *Giardia* infection, children living in Benue, Kano, Katsina, Ondo, and Plateau states had significantly lower odds of infection, with reductions of 78%, 69%, 67%, 94%, and 89%, respectively, compared to those in Borno state. Similar to *Cryptosporidium*, drinking well water was significantly associated with an increased likelihood of *Giardia* infection (OR = 1.68, P = 0.04, CI = 1.03–2.74) compared to borehole water. Sheep domestication was also significantly associated with lower odds of *Giardia* infection (OR = 0.46, P = 0.05, CI = 0.21–1.01). Coinfection with *Cryptosporidium* and *Entamoeba* increased the odds of *Giardia* infection, with odds ratios of 6.01 and 4.71, respectively (Table 4).

For *Entamoeba* infection, males had significantly higher odds of infection compared to females (OR = 1.61, P = 0.03, CI = 1.06–2.46). Living in Cross River State was associated with a significantly increased likelihood of amebiasis, with children in Cross River being 4.24 times more likely to be infected compared to those in Borno State (OR = 4.24, P = 0.02, CI = 1.30–15.61). Children from households where the head of the household was an artisan, farmer, or trader had significantly higher odds of *Entamoeba* infection compared to those with civil servant heads of household. In contrast to *Cryptosporidium* and *Giardia*, drinking tap water significantly increased the odds of *Entamoeba* infection compared to drinking borehole water (OR = 6.31, P = 0.00, CI = 1.90–21.94). Washing vegetables was also significantly associated with higher odds of *Entamoeba* infection (OR = 1.87, P = 0.04, CI = 1.02–3.44). Coinfection with *Giardia* was associated with a significantly higher likelihood of *Entamoeba* infection (OR = 4.62, P = 0.00, CI = 2.23–10.92) (Table 4).

The multivariate logistic regression analysis for coinfections involving two or all three parasites identified several significant risk factors. These included state (except for *Cryptosporidium + Entamoeba* coinfection), occupation (*Giardia + Entamoeba* only), household density (*Cryptosporidium + Giardia* and *Cryptosporidium + Giardia + Entamoeba* coinfections), water source (all coinfection categories), washing of

	Cryptosporidium			*Giardia*			*Entamoeba*		
	aOR	P	CI (95%)	aOR	P	CI (95%)	aOR	P	CI (95%)
Gender									
Female	Ref			Ref			Ref		
Male	0.87	0.47	0.61–1.26	0.91	0.61	0.65–1.29	1.61	**0.03**	1.06–2.46
State									
Borno	Ref			Ref			Ref		
Benue	0.61	0.42	0.19–2.02	0.22	**0.01**	0.06–0.72	0.78	0.76	0.16–3.83
Cross River	0.43	0.13	0.14–1.26	0.44	0.13	0.14–1.26	4.24	**0.02**	1.30–15.61
Edo	0.44	0.26	0.11–1.82	1.17	0.84	0.25–5.85	0.82	0.83	0.14–4.85
Enugu	1.02	0.97	0.35–3.05	0.39	0.12	0.11–1.24	0.68	0.60	0.17–3.00
Jigawa	0.39	0.18	0.10–1.51	0.55	0.42	0.13–2.35	2.22	0.33	0.45–11.64
Kano	0.14	**0.00**	0.04–0.45	0.31	**0.04**	0.10–0.90	3.13	0.10	0.84–12.97
Katsina	1.60	0.32	0.63–4.11	0.33	**0.04**	0.11–0.93	2.92	0.09	0.87–10.92
Ondo	1.29	0.71	0.33–4.82	0.06	**0.00**	0.02–0.18	0.25	0.25	0.01–2.03
Plateau	0.22	**0.02**	0.06–0.80	0.11	**0.00**	0.04–0.32	1.03	0.97	0.20–5.03
Head of household education level									
Tertiary	Ref			Ref			Ref		
Informal	3.18	0.11	0.83–14.37	0.57	0.33	0.18–1.74	1.08	0.93	0.25–5.82
Primary	2.15	0.28	0.57–9.52	0.83	0.74	0.27–2.48	1.34	0.70	0.32–7.21
Secondary	1.82	0.38	0.50–7.86	0.69	0.49	0.23–1.95	1.84	0.42	0.47–9.57
Occupation									
Civil service	Ref			Ref			Ref		
Artisanal	0.60	0.30	0.23–1.55	0.93	0.86	0.42–2.08	5.14	**0.00**	1.85–14.72
Farming	0.49	0.12	0.20–1.18	0.85	0.69	0.39–1.86	3.66	**0.01**	1.33–10.31
Self employment	1.73	0.41	0.47–6.38	0.82	0.80	0.18–4.62	1.27	0.85	0.05–10.96
Skilled worker	0.15	0.09	0.01–0.91	0.68	0.47	0.24–1.98	1.94	0.35	0.44–7.40
Trading	0.61	0.15	0.31–1.20	1.09	0.78	0.59–2.03	4.48	**0.00**	1.93–10.83
SES									
Med	Ref			Ref			Ref		
Low	0.65	0.56	0.14–2.58	1.15	0.81	0.37–3.74	0.44	0.31	0.08–1.95
Type of housing									
Private	Ref			Ref			Ref		
Shared	0.78	0.25	0.51–1.2	1.43	0.10	0.93–2.19	0.76	0.29	0.46–1.27
Household density									
Low	Ref			Ref			Ref		
High	1.55	0.17	0.84–2.99	1.55	0.08	0.94–2.53	0.60	0.13	0.32–1.18
Symptomology									
No	Ref			Ref			Ref		
Yes	0.65	0.14	0.36–1.15	0.67	0.12	0.40–1.11	0.64	0.21	0.32–1.27
Water source									
Borehole	Ref			Ref			Ref		
Sachet water	0.84	0.58	0.44–1.55	0.99	0.97	0.59–1.69	2.00	0.06	0.96–4.12
Stream	0.88	0.87	0.17–4.11	1.66	0.42	0.48–5.70	4.69	0.31	0.16–137.36
Tap water	2.70	0.10	0.82–8.91	0.64	0.49	0.17–2.36	6.31	**0.00**	1.90–21.94
Well	1.78	**0.04**	1.02–3.14	1.68	**0.04**	1.03–2.74	1.23	0.52	0.66–2.30
Water treatment									
No	Ref			Ref			Ref		
Yes	0.78	0.43	0.42–1.42	1.06	0.85	0.61–1.84	0.85	0.66	0.39–1.72
Type of toilet									
Water closet	Ref			Ref			Ref		
Bush	1.00	1.00	0.46–2.10	1.78	0.19	0.78–4.41	1.00	0.99	0.30–2.74
Pit latrine	0.54	**0.02**	0.32–0.90	0.97	0.90	0.63–1.51	0.98	0.94	0.57–1.68
Handwashing									
No	Ref			Ref			Ref		
Yes	0.73	0.34	0.39–1.40	1.01	0.97	0.48–2.01	1.00	1.00	0.44–2.52
Wash vegetables									
Continued									

	Cryptosporidium			*Giardia*			*Entamoeba*		
	aOR	P	CI (95%)	aOR	P	CI (95%)	aOR	P	CI (95%)
No	Ref			Ref			Ref		
Yes	1.67	**0.04**	1.03–2.72	0.83	0.46	0.51–1.36	1.87	**0.04**	1.02–3.44
Living with animals									
No	Ref			Ref			Ref		
Cattle	1.11	0.85	0.37–3.06	1.44	0.55	0.47–5.10	0.79	0.67	0.25–2.27
Goat	1.12	0.75	0.54–2.29	0.59	0.11	0.31–1.14	0.86	0.72	0.36–1.98
Sheep	1.78	0.18	0.76–4.08	0.46	**0.05**	0.21–1.01	0.57	0.21	0.23–1.34
Fowl	2.35	**0.01**	1.27–4.37	0.75	0.36	0.40–1.40	0.75	0.51	0.32–1.71
Cat	2.74	0.08	0.83–8.41	1.30	0.72	0.33–6.59	0.93	0.93	0.13–4.08
Dog	1.58	0.16	0.83–2.98	0.76	0.41	0.40–1.46	0.87	0.76	0.34–2.09
Other infections									
No	Ref			Ref			Ref		
Cryptosporidium	NA	NA	NA	6.01	**0.00**	3.09–12.99	1.34	0.29	0.77–2.27
Giardia	5.87	**0.00**	3.03–12.64	NA	NA	NA	4.62	**0.00**	2.23–10.92
Entamoeba	1.39	0.23	0.8–2.35	4.71	**0.00**	2.27–11.19	NA	NA	NA

Table 4. Logistic regression analysis of risk factors associated with *Cryptosporidium, Giardia* and *Entamoeba* infections. *OR* Odds Ratio, Bolded values are significant at P = 0.05.

vegetables (except *Cryptosporidium* + *Entamoeba* coinfection), and living with animals (*Cryptosporidium* + *Giardia* and *Cryptosporidium* + *Giardia* + *Entamoeba* coinfections) (Table 5).

For coinfections involving *Cryptosporidium* + *Giardia*, children living in Kano (OR = 0.12, P = 0.00, CI = 0.04–0.37) and Plateau (OR = 0.08, P = 0.00, CI = 0.02–0.32) had significantly lower odds of being coinfected with both parasites compared to those residing in Borno. Conversely, the likelihood of coinfection significantly increased with higher household density (OR = 2.02, P = 0.03, CI = 1.08–4.01), drinking well water (OR = 1.74, P = 0.05, CI = 1.00–3.06), and washing vegetables (OR = 1.71, P = 0.03, CI = 1.05–2.78). Additionally, those living with fowls (OR = 2.74, P = 0.00, CI = 1.33–4.63) or cats (OR = 3.54, P = 0.03, CI = 1.05–11.13) had a significantly higher likelihood of being coinfected with both *Cryptosporidium* and *Giardia* (Table 5).

In the case of *Giardia* + *Entamoeba* coinfection, only residents of Cross River State showed significantly higher odds of being coinfected with both parasites compared to those in Borno (OR = 3.65, P = 0.04, CI = 1.13–13.25). Occupation type was also significantly associated with *Giardia* + *Entamoeba* coinfection, with artisans, farmers, and traders being three to four times more likely to be coinfected (OR = 3.95, P = 0.01, CI = 1.39–11.53; OR = 3.17, P = 0.03, CI = 1.14–9.13 & OR = 4.19, P = 0.00, CI = 1.79–10.33). Similar to the *Cryptosporidium* + *Giardia* coinfections, drinking tap water and washing vegetables significantly increased the odds of *Giardia* + *Entamoeba* coinfections (OR = 5.40, P = 0.01, CI = 1.63–18.63 & OR = 1.84, P = 0.05, CI = 0.99–3.44) (Table 5).

For *Cryptosporidium* + *Entamoeba* coinfection, drinking either tap or well water significantly increased the likelihood of coinfection (OR = 86.84, P = 0.00, CI = 7.55–1626.09 & OR = 5.31, P = 0.02, CI = 1.37–22.84). No other significant risk factors were identified for this coinfection (Table 5).

Coinfection with all three parasites (*Cryptosporidium, Giardia*, and *Entamoeba*) was significantly influenced by water sources. The odds of coinfection significantly increased with drinking tap (OR = 50.42, P = 0.00, CI = 5.10–714.85) or well water (OR = 4.39, P = 0.05, CI = 1.06–19.61).

Discussion

Intestinal protozoa, such as *Cryptosporidium*, *Entamoeba*, and *Giardia* species, are significant causes of diarrhoea in children in sub-Saharan Africa. Despite their public health significance, limited attention has been given to these infections among children in Nigeria. Previous studies on these pathogens primarily relied on microscopy, which has limited sensitivity. This study provides the first nationwide assessment of the prevalence of *Cryptosporidium*, *Giardia*, and *Entamoeba* in Nigerian children aged 10 years and below, using real-time PCR, a more sensitive and specific diagnostic approach.

The study found an overall prevalence of *Cryptosporidium* (18.1%), *Giardia* (77.6%), and *Entamoeba* (12.3%) species, which contrasts with findings from Tanzania and Colombia, where lower prevalences were reported[20,21]. Similarly, Dagne and Alelign[22] observed lower prevalences of *Cryptosporidium* and *Giardia* in Ethiopia but reported a higher prevalence of *Entamoeba* species. In terms of coinfection, the prevalence of *Cryptosporidium* + *Giardia* coinfection was notably higher than those reported in previous studies from Nigeria, Colombia, and Egypt[18,21,23,24]. Similar increases in coinfection frequency were noted for *Giardia* + *Entamoeba* compared to studies by Haliu and Alemu[25] and Atu et al.[18] in Ethiopia and Nigeria, respectively. It however differed from the report by Tijani et al.[26]. Furthermore, the findings of *Cryptosporidium* + *Entamoeba* coinfection in this study differed from earlier reports by Atu et al.[18]. The frequency of triple coinfection with all three parasites in this study was consistent with that observed by Atu et al.[18] in Nigeria but diverged from the results reported by Ibrahim et al.[24] in Egypt.

	Cryptosporidium + Giardia			*Giardia + Entamoeba*			*Cryptosporidium + Entamoeba*			*Cryptosporidium + Giardia + Entamoeba*		
	aOR	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**
Gender												
Female	Ref			Ref			Ref			Ref		
Male	0.86	0.43	0.60–1.24	1.52	0.06	0.99–2.35	1.15	0.74	0.49–2.72	1.35	0.50	0.57–3.25
State												
Borno	Ref			Ref			Ref			Ref		
Benue	0.42	0.15	0.13–1.38	0.62	0.56	0.13–3.07	0.11	0.13	0.01–1.98	0.12	0.16	0.01–2.24
Cross River	0.40	0.10	0.13–1.17	3.65	**0.04**	1.13–13.25	0.82	0.87	0.06–9.90	0.90	0.93	0.07–10.18
Edo	0.42	0.22	0.10–1.71	0.77	0.78	0.13–4.64	0.38	0.58	0.01–11.16	0.37	0.56	0.01–11.29
Enugu	0.84	0.75	0.29–2.49	0.56	0.43	0.13–2.50	0.66	0.75	0.06–9.38	0.60	0.69	0.05–8.28
Jigawa	0.33	0.10	0.09–1.24	2.27	0.32	0.46–11.71	0.09	0.15	0.00–2.29	0.12	0.18	0.00–2.60
Kano	0.12	**0.00**	0.04–0.37	2.76	0.14	0.73–11.48	0.26	0.33	0.02–4.02	0.30	0.38	0.02–4.62
Katsina	1.07	0.89	0.43–2.73	2.33	0.18	0.70–8.67	0.71	0.76	0.08–7.25	0.68	0.72	0.08–6.71
Ondo	0.58	0.41	0.15–2.08	0.16	0.13	0.01–1.27	NA	NA	NA	NA	NA	NA
Plateau	0.08	**0.00**	0.02–0.32	0.15	0.11	0.01–1.19	0.26	0.40	0.01–5.38	NA	NA	NA
Head of household education level												
Tertiary	Ref			Ref			Ref			Ref		
Informal	2.2	0.26	0.59–9.74	0.66	0.60	0.15–3.61	0.37	0.50	0.02–11.78	0.30	0.43	0.02–9.82
Primary	1.48	0.57	0.41–6.41	1.07	0.93	0.26–5.76	0.34	0.48	0.02–11.42	0.25	0.36	0.01–8.26
Secondary	1.28	0.71	0.37–5.39	1.29	0.74	0.32–6.70	0.71	0.81	0.05–21.97	0.42	0.56	0.03–13.08
Occupation												
C. Service	Ref			Ref			Ref			Ref		
Artisanal	0.47	0.12	0.18–1.21	3.95	**0.01**	1.39–11.53	4.51	0.19	0.48–50.89	2.47	0.46	0.22–30.27
Farming	0.43	0.06	0.18–1.01	3.17	**0.03**	1.14–9.13	1.47	0.74	0.16–16.70	1.32	0.81	0.14–14.70
S. Employed	1.25	0.73	0.35–4.40	1.11	0.93	0.05–9.00	NA	NA	NA	NA	NA	NA
S. Worker	0.15	0.08	0.01–0.88	1.21	0.80	0.23–5.01	NA	NA	NA	NA	NA	NA
Trading	0.56	0.09	0.29–1.09	4.19	**0.00**	1.79–10.33	4.84	0.10	0.85–40.39	4.28	0.13	0.77–37.10
SES												
Med	Ref			Ref			Ref			Ref		
Low	0.86	0.83	0.19–3.27	0.64	0.58	0.11–2.82	1.43	0.82	0.04–23.74	2.21	0.61	0.06–39.12
Housing type												
Private	Ref			Ref			Ref			Ref		
Shared	0.85	0.44	0.55–1.3	0.86	0.58	0.52–1.45	0.53	0.19	0.20–1.37	0.57	0.25	0.22–1.47
Household density												
Low	Ref			Ref			Ref					
High	2.02	**0.03**	1.08–4.01	0.64	0.19	0.33–1.28	3.99	0.21	0.67–78.46	3.51	0.25	0.60–68.05
Symptomology												
No	Ref			Ref			Ref				Ref	
Yes	0.59	0.08	0.33–1.05	0.63	0.19	0.31–1.25	0.64	0.52	0.16–2.34	0.71	0.62	0.17–2.65
Water source												
Borehole	Ref			Ref			Ref			Ref		
Sachet water	0.75	0.37	0.39–1.40	2.01	0.07	0.93–4.21	1.34	0.73	0.23–6.55	1.53	0.60	0.27–7.16
Stream	0.50	0.46	0.06–2.74	6.12	0.23	0.22–172.41	NA	NA	NA	NA	NA	NA
Tap water	2.39	0.13	0.76–7.51	5.40	**0.01**	1.63–18.63	86.84	**0.00**	7.55–1626.09	50.42	**0.00**	5.10–714.85
Well	1.74	**0.05**	1.00–3.06	1.39	0.31	0.74–2.64	5.31	**0.02**	1.37–22.84	4.39	**0.05**	1.06–19.61
Water treatment												
No	Ref			Ref			Ref			Ref		
Yes	0.90	0.72	0.49–1.60	1.04	0.91	0.49–2.12	2.13	0.22	0.60–6.98	2.47	0.14	0.72–8.02
Toilet type												
Water closet	Ref			Ref			Ref			Ref		
Bush	1.33	0.45	0.61–2.78	0.87	0.82	0.24–2.52	0.62	0.68	0.03–4.08	0.68	0.73	0.03–4.43
Pit latrine	0.64	0.08	0.39–1.05	0.94	0.83	0.55–1.62	0.39	0.10	0.12–1.16	0.41	0.12	0.13–1.23
Handwashing												
No	Ref			Ref						Ref		
Yes	0.92	0.80	0.49–1.80	1.12	0.80	0.48–2.98	NA	NA	NA	NA	NA	NA
Wash vegetables												
Continued												

	Cryptosporidium + Giardia			*Giardia + Entamoeba*			*Cryptosporidium + Entamoeba*			*Cryptosporidium + Giardia + Entamoeba*		
	aOR	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**	**aOR**	**P**	**CI (95%)**
No	Ref			Ref			Ref			Ref		
Yes	1.71	**0.03**	1.05–2.78	1.84	**0.05**	0.99–3.44	2.10	0.22	0.65–7.34	2.41	0.15	0.75–8.64
Living with animals												
No	Ref			Ref			Ref			Ref		
Cattle	1.35	0.57	0.45–3.69	0.70	0.54	0.21–2.10	0.76	0.78	0.08–4.56	0.77	0.79	0.08–4.54
Goat	1.18	0.65	0.58–2.36	0.83	0.67	0.35–1.91	1.95	0.40	0.41–9.34	1.77	0.46	0.38–7.96
Sheep	1.41	0.42	0.60–3.20	0.50	0.12	0.20–1.18	0.39	0.28	0.06–1.98	0.44	0.33	0.07–2.14
Fowl	2.47	**0.00**	1.33–4.63	0.82	0.65	0.35–1.89	1.88	0.41	0.42–8.47	1.83	0.44	0.38–8.46
Cat	3.54	**0.03**	1.05–11.13	0.48	0.51	0.02–2.85	NA	NA	NA	NA	NA	NA
Dog	1.5	0.21	0.79–2.83	1.04	0.94	0.41–2.47	0.58	0.54	0.07–2.97	0.70	0.69	0.09–3.59
Other infections												
No	Ref			Ref			Ref			Ref		
Cryptosporidium	NA	NA	NA	1.65	0.07	0.95–2.82	NA	NA	NA	NA	NA	NA
Giardia	NA	NA	NA	NA	NA	NA	7.24	0.06	1.35–137.32	NA	NA	NA
Entamoeba	1.67	0.06	0.96–2.83	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 5. Logistic regression analysis of risk factors associated with *Cryptosporidium, Giardia* and *Entamoeba* coinfections.

The higher prevalences of both single and coinfections observed in this study may primarily be attributed to the use of real-time PCR, which offers significantly greater sensitivity compared to traditional microscopy or standard PCR techniques. Similar findings have been reported in previous studies that compared real-time PCR with microscopy[21]. Molecular methods allow for the simultaneous detection of multiple parasites in a single stool sample, eliminating the need for multiple stool examinations[27].

The unexpectedly high prevalence of *Giardia* infection across all study sites, along with the strong correlation between *Entamoeba*-only infections and *Giardia + Entamoeba* coinfections ($r = 0.99$, $p < 0.0000001$), suggests that *Giardia* may influence the spectrum of diarrheal disease in these regions. Asymptomatic *Giardia* infections are increasingly recognized in seemingly healthy children in low- and middle-income countries (LMICs), with evidence suggesting they may offer some protection against diarrheal diseases[28,29]. Several studies have proposed that *Giardia* infection may either confer protection against diarrheal disease or act as an opportunistic pathogen, increasing susceptibility to other diarrheal agents[30,31].

A recent study by Sardinha-Silva et al.[29] supports the former hypothesis, suggesting that *Giardia* infection may provide mutualistic protection against inflammatory processes by modulating mucosal immunity toward a Type 2 response, which is associated with a reduced risk of severe diarrhoea. In contrast, pathogens such as *Entamoeba* and certain viruses that elicit strong proinflammatory responses, may lead to gut damage and severe diarrheal outcomes. Type 2 immunity likely acts to counterbalance this excessive inflammation, potentially mitigating the severity of coinfections. In *Entamoeba*-endemic regions, the presence of *Giardia* may therefore play a protective or modulatory role in host immune responses to proinflammatory infections[29]. Natural selection or ecological interactions may favour the persistence of *Giardia* in such settings, particularly if its presence results in milder disease outcomes when coinfections occur.

In this study, we observed significant variations in the prevalence of single and co-infections of the studied parasites in relation to sociodemographic characteristics of the population. Regarding age, we found that cryptosporidium infection was most prevalent in children aged 5–10 years. This finding contrasts with earlier reports suggesting that cryptosporidiosis is highly prevalent in early childhood, with most children being infected by the age of 2[8]. Most studies on the epidemiology of cryptosporidiosis focus on children under five years old, resulting in limited data for older age groups. However, our observation aligns with findings by Efunshile et al.[32] and Atu et al.[23] in Nigeria, who also reported a higher prevalence of cryptosporidiosis among children aged 6–10 years compared to younger children. While the reasons for this discrepancy across age groups are unclear, children under 10 are more prone to poor hygiene practices, increased water exposure, and the tendency to eat with unwashed hands or consume contaminated fruits and vegetables without adult supervision, all of which predispose them to enteric diseases[33,34]. These findings emphasize the need for further research into the epidemiology of cryptosporidiosis in older children. In terms of gender, males were found to be significantly more likely to be infected with *Entamoeba* than females. Previous studies have suggested that males, across all age groups, are more susceptible to invasive amebiasis[35], potentially due to differences in behaviour or personal hygiene. This pattern has also been observed in studies conducted in Nigeria and Kenya[34,36]. Additionally, our study revealed a significant association between parental education level and *Giardia* infection. Specifically, children whose parents had tertiary education showed a higher prevalence of *Giardia* infections. This contrasts with the commonly reported trend that higher parental education levels are associated with lower rates of gastrointestinal parasitic infections[37,38]. Literate parents are generally expected to practice better hygiene, which reduces the risk of infection. However, in this case, children with educated parents might be exposed to secondary contamination from infected daycare staff, nannies, or food handlers[39]. Furthermore, we found a significantly higher infection prevalence in children of traders for single infections of *Cryptosporidium*, *Giardia*, and *Entamoeba*, as well as in co-infections of *Cryptosporidium* + *Giardia* and *Giardia* + *Entamoeba*. This could be linked to the consumption of inadequately washed fruits and vegetables from the market. A review by Eslahi et al.[40] on the link between fruits, vegetables, and protozoan parasites found that Nigeria had the highest contamination rates of fruits and vegetables with protozoan parasites. A study by Kanya et al.[41] also reported that occupation influenced *Cryptosporidium* infection rates in Kebbi state, although this contrasts with findings by Tombang et al.[42] in Cameroon, where no significant association between *Cryptosporidium* infection and parental occupation was observed. Similarly, while Ogbuu et al.[43] found a significant association between parental occupation and *Giardia* infection in Nigeria, this was not supported by the findings of Efunshile et al.[32], who reported no significant link between giardiasis prevalence and father's occupation. Finally, the findings of Victor et al.[44], which showed no significant association between *Entamoeba* infection and caregiver occupation, differ from the results of our study. The prevalence of *Entamoeba* infection varied significantly with the socioeconomic status of the study population. This finding aligns with previous reports suggesting that low socioeconomic status is a major risk factor for intestinal parasitic infections[45]. In Nigeria, individuals living in poor socioeconomic conditions commonly report *Entamoeba* infections[46,47]. Similarly, a study conducted in Kenya found that individuals from households with the lowest socioeconomic status were at the greatest risk of infection with *E. histolytica/dispar*[48].

This study also identified statistically significant risk factors for both single and co-infections with the various parasites investigated. The most common risk factors were geographic location, source of water and coinfection with other parasites. In the case of *Cryptosporidium*, recent reviews highlighted crowded living conditions, animal contact, location, drinking underground or tap water, eating unwashed fruits and open defecation as major contributors to *Cryptosporidium* infections in low- and middle-income countries (LMICs)[14,49]. These findings partially align with our results, which identified contact with fowl as a significant risk factor for cryptosporidiosis. Interestingly, Kano and Plateau States were significantly associated with lower odds of *Cryptosporidium* infection. According to the Federal Ministry of Health[50], residents of Kano had higher access to WASH (Water, Sanitation, and Hygiene) services compared to the reference state, which may partly explain the lower infection rates observed. However, no clear explanation was found for the reduced risk observed in Plateau State. The risk of cryptosporidiosis was notably higher among individuals who relied on well water for drinking ($p = 0.04$). This finding is consistent with existing knowledge, as *Cryptosporidium* is commonly transmitted through faecal contamination of water and food[51,52]. According to UNICEF, while approximately 67% of Nigerians have access to basic drinking water, more than half of these improved water sources are contaminated with pathogens[50]. Similar patterns have been reported globally in both field studies and outbreak investigations, where groundwater systems have been implicated as major sources of enteric pathogens[53]. Numerous studies have documented the contamination of well water with *Cryptosporidium* in various parts of the world[54–56], across Africa[57], and specifically in Nigeria[58,59]. The observed lower risk of cryptosporidiosis among pit latrine users compared to water closet users contrasts with the review by Bouzid et al.[49], who reported that open defecation is commonly associated with a higher prevalence of *Cryptosporidium* infections in LMICs. However, the use of public water closet facilities has also been identified as a significant risk factor for *Cryptosporidium* infection[60]. In Nigeria, evidence regarding the relationship between toilet type and cryptosporidiosis remains inconsistent. Some studies have reported higher prevalence rates among water closet users[61], while others found no significant association between *Cryptosporidium* infection and toilet type[62,63]. The increased risk observed among water closet users in the present study may be attributed to the sharing of toilet facilities by multiple persons or households, which often results in poor hygiene and maintenance. Similar observations have been reported by Enitan et al.[64] in south-western Nigeria. The increased risk of cryptosporidiosis among individuals who reported washing vegetables aligns with the findings of Bunza et al.[65], but contrasts with those of Dankwa et al.[60]. Although this observation deviates from the typical epidemiological pattern, it is not entirely unexpected, given the well-established link between water quality and parasitic contamination[14,66]. The use of contaminated water sources for washing vegetables elevates the risk of infection, which likely explains the findings in this study. Several studies in Nigeria have reported microbial contamination of vegetables, and conventional washing with water alone may be insufficient to eliminate *Cryptosporidium* oocysts[67,68]. Moreover, individuals in this group may have also been exposed to other potential sources of *Cryptosporidium* infection. In this study, contact with animals was significantly associated with infections by *Cryptosporidium*. Similar associations have been reported in previous studies[14,22]. Specifically, the risk of cryptosporidiosis was found to increase with contact with fowl ($p = 0.01$). *Cryptosporidium* infection has been documented in poultry across Africa[69–72], and this represents a significant public health concern, particularly due to the common practice of rearing poultry for household consumption in Nigeria. Zoonotic species such as *C. meleagridis* have been identified in both poultry and humans[73,74], further underscoring the potential for transmission. The risk of cryptosporidiosis in this study increased significantly in individuals co-infected with *Giardia*. Co-infections of *Cryptosporidium* and *Giardia* have been reported in several studies across Nigeria and other parts of Africa[14,21,23,24]; however, there remains a paucity of research on the potential interactions between these two parasites. The observed risk pattern may be attributed to similarities in their modes of transmission and sites of infection. Both parasites are primarily transmitted via contaminated food and water and predominantly colonize the small intestine[75].

Regarding *Giardia*, a lack of safe drinking water, inadequate access to sanitation facilities, and age have been reported as global risk factors for giardiasis. In Africa, additional risk factors identified include contact with animals or manure, locality, and the consumption of unwashed or raw fruits and vegetables[14,76]. These findings partially align with the results of this study. Interestingly, five states demonstrated a reduced risk of *Giardia* infection. While Kano and Katsina states benefit from relatively higher access to Water, Sanitation, and Hygiene (WASH) services, according to the Federal Ministry of Health (FMOH), the reasons for the reduced risk observed in Plateau, Benue, and Ondo states remain unclear[50]. As with *Cryptosporidium*, the risk of giardiasis was found to increase with the use of well water ($p = 0.04$). This is consistent with previous reports documenting

Giardia contamination in wells across Nigeria and Africa, often linked to environmental pollution from refuse dumps, erosion, surface runoff, and the use of unprotected wells[59,77,78]. Interestingly, rearing sheep appeared to be protective against giardiasis in this dataset ($p = 0.05$), although the association was only marginally significant. The observed association may reflect potential zoonotic exposure rather than a protective effect. *Giardia* assemblage E, traditionally considered host-specific and predominant in grazing or herd animals, has recently been shown to also infect humans.[79]. However, there is currently a paucity of data on *Giardia* infections in sheep in Nigeria, limiting conclusive interpretations. More notably, a higher risk of giardiasis was associated with co-infection by *Cryptosporidium* and *Entamoeba* in this study suggesting that *Giardia* may play a protective role against acute diarrhoea and could serve as an environmental marker for exposure to multiple intestinal pathogens[80].

With respect to *Entamoeba* infections, existing literature identifies several demographic and socioeconomic factors (age, gender, occupation, income, family size, nutritional status, animal contact), host genetic and immune factors, as key risk determinants[17]. In this study, males were found to have a higher risk of infection with *Entamoeba* ($p = 0.03$) which does not align with previous reports suggesting the probability of exposure is similar across genders since *E. histolytica* is transmitted via ingestion of contaminated food or water[3]. Invasive disease tends to be more common in adult males, likely due to immunological differences between sexes[35,81–85]. In contrast to *Cryptosporidium* and *Giardia*, the risk of *Entamoeba* infection was significantly higher in Cross River State ($p = 0.02$). This finding aligns with previous studies that have documented the endemicity of *Entamoeba* infections in the region, largely attributed to inadequate environmental sanitation and poor personal hygiene practices[83,86,87]. There are conflicting reports regarding the association between occupation and intestinal parasitic infections in Nigeria[83,88,89]. However, our findings are consistent with those of Dagne and Alelign[22] and Roro et al.[90], who found that children from farming families were two to seven times more likely to be infected ($p = 0.01$). This increased risk is attributed to frequent contact with manure and poor hygiene practices, especially among children who accompany their parents to farms. Similarly, trading has been identified as a significant occupational risk factor for *Entamoeba* infections in Kenya[91]. The occupational risk observed in this study may also reflect broader exposures to environmental contamination and inadequate sanitation. Conversely, a study in Ghana found no significant association between occupation and infection risk[45]. The association between lack of access to safe drinking water and increased risk of *Entamoeba* infection has been well established[17,92–94]. A study in Cameroon by Nsoh et al.[57] identified eight species of enteric protozoa, including *Entamoeba*, in tap water. In Nigeria, several studies have similarly linked *Entamoeba* risk to the consumption of untreated water[82,89]. In addition, this study found a higher risk of *Entamoeba* infection among individuals who reported washing vegetables ($p = 0.04$), and the use of tap water ($p = 0.00$). The elevated risk of *Entamoeba* infection among those already infected with *Giardia* may be due to co-contamination and shared transmission routes.

This study also identified several risk factors associated with coinfections involving the three parasites. The observed polyparasitism may be attributed to shared risk factors such as the fecal–oral transmission route, poor sanitation and hygiene practices, and low socio-economic conditions. For coinfection with *Cryptosporidium* and *Giardia*, similar to single *Cryptosporidium* infections, individuals in Kano and Plateau States had significantly lower odds of infection ($p = 0.00$). Although the reasons for this are not immediately clear, it may be due to a combination of economic and environmental factors, including access to potable water and WASH services. An increased risk of coinfection was observed in households with higher population densities. This finding aligns with prior research indicating human-to-human transmission of parasitic protozoans[95]. According to reports[14,96], children who had contact with family members exhibiting gastrointestinal symptoms are particularly at risk. The significant association between high household density and *Cryptosporidium* + *Giardia* coinfection suggests that children living in larger households may be more susceptible to dual infections, consistent with previous studies[45,64,97,98]. Drinking well water was also significantly associated with coinfection ($p = 0.05$), which is unsurprising given that it was a risk factor for single infections with both parasites in this study. In sub-Saharan Africa, *Cryptosporidium* and *Giardia* are prevalent in water sources due to multiple routes of contamination (human and animal feaces) and their resistance to common water disinfectants. These parasites can survive in water for up to a year, making residents in rural areas with inadequate water treatment and poor hygiene practices continuously vulnerable[99,100]. Expectedly, a higher risk of infection was observed among individuals who reported washing vegetables ($p = 0.03$), potentially due to the use of contaminated water. In many rural African households, untreated water is used for rinsing produce, facilitating foodborne transmission of these parasites. Moreover, the growing trend of consuming raw or undercooked vegetables to retain taste and preserve nutrients may further increase exposure risk[14,101]. A significantly higher risk of coinfections was associated with contact with both fowls and cats ($p = 0.00/0.03$). Similar to *Cryptosporidium*, several studies have reported varying prevalences of *Giardia* infections in domestic poultry in Nigeria[102–104]. Cats are recognized as potential hosts for both *Cryptosporidium* and *Giardia*, but the zoonotic transmission risk to humans remains uncertain[105,106]. Some studies suggest that the likelihood of zoonotic transmission may be low, depending on the specific subtypes involved[106,107]. While data on feline cryptosporidiosis in Nigeria are limited, *Giardia* infection has been documented in domestic cats, with reported prevalence rates of up to 5%[108]. Close contact with domestic, farmed, or companion animals has been suggested to increase the risk of transmission of both *Giardia* and *Cryptosporidium*[37,109,110].

Except for gender and infection with *Giardia*, all risk factors associated with single *Entamoeba* infections were also significantly associated with *Giardia* and *Entamoeba* coinfections suggesting that the coinfection pattern may be primarily *Entamoeba*-driven. *Giardia* and *Entamoeba* coinfections were more common in states with high *Entamoeba* prevalence. The use of well or tap water was the only significant risk factor associated with *Cryptosporidium* and *Entamoeba* coinfection ($p = 0.00/0.02$). These water sources were also implicated in single infections with each parasite. Notably, the odds ratios for coinfections were very large with wide confidence intervals, likely reflecting sparse data in exposure–infection combinations. These findings should therefore be interpreted with caution. Nevertheless, in many parts of Africa, water sources are frequently contaminated with one or more waterborne protozoa, making coinfections common among individuals who rely on them for drinking. Additionally, climatic changes, particularly drought, have been proposed to exacerbate the spread of waterborne parasitic diseases in the region[99].

The risk of coinfection with all three parasites in this study was significantly associated with the use of tap or well water ($p = 0.00/0.05$). Notably, the association with tap water was both strong and statistically significant, with individuals using tap water being over 50 times more likely to be infected (OR = 50.42). However, the wide confidence interval (95% CI 5.10–714.85) suggests variability in the estimate, likely due to a small number of infected individuals using tap water. While the magnitude of this effect should be interpreted with caution, the marginal to strong statistically significant associations observed between water sources and the various parasitic infections align with the typical transmission patterns of parasitic protozoa. These findings indicate that contaminated water sources may be the primary contributor to multiple coinfections in this population.

The notably high prevalence of *Giardia* and the observed association with *Entamoeba sp.* infection rates in this dataset raises important questions about the nature of co-infections. The findings suggest that no single mechanism fully explains the observed pattern. The high prevalence of *Giardia* across all study locations and its strong correlation with *Entamoeba* coinfections may be best explained by a combination of shared transmission pathways and biological interactions. It is likely that contaminated food, water, or common sanitation practices have contributed to the co-transmission of *Giardia* and *Entamoeba* and/or *Giardia* and *Cryptosporidium* in these settings[14]. Biological interactions are also possible, but remain speculative. Emerging evidence suggest that *Giardia* may modulate host immune responses, potentially influencing susceptibility to other enteric pathogens[29]. However, this study did not assess immunological outcomes, so any protective or modulatory role of *Giardia* remains hypothetical. However, these findings generate hypotheses for future research. Studies incorporating immunological profiling and environmental surveillance are needed to disentangle the relative contributions of environmental exposure and potential biological interactions in shaping co-infection patterns.

The main strengths of this study include the use of molecular diagnostic techniques and a relatively large sample size, covering ten states across different ecological zones in Nigeria. However, there are notable limitations. First, species typing was not performed, which would have provided valuable information on the circulating strains in different regions. Importantly, the species of *Entamoeba* infection was not resolved, and the pathogenicity of infection is significantly dependent on the *Entamoeba* species present, limiting the interpretation of the clinical significance of the findings. Second, some responses obtained through the epidemiological questionnaire may be subject to recall or reporting bias, particularly as they were provided by children, potentially affecting the accuracy of the statistical analyses. Thirdly, qPCR is highly sensitive and may identify low-level infections, asymptomatic carriage, or residual parasite DNA that does not necessarily reflect active clinical disease. Therefore, some of the positive results observed in this study may represent low-intensity colonization or transient exposure.

Conclusion

This study presents the most up-to-date and comprehensive report on the epidemiology of three significant parasitic protozoa in Nigeria. Our findings confirm that *Cryptosporidium*, *Entamoeba*, and *Giardia* species are prevalent across the country. There is an urgent need to improve access to potable water and strengthen WASH (Water, Sanitation, and Hygiene) services nationwide to curb the transmission of these parasites.

Data availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Author contributions

A.O.G. and G.M.E. conceptualized the study. A.O.G., A.E.E., A.M., A.U.M., A.T., B.J., D.A.S., E.E.O., N.V.S., O.A., conducted field surveys. A.O.G. analysed the data and drafted the manuscript. G.M.E. reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

Ethical approval was obtained from the ethics committees of the State Ministries of Health and Hospital Management Boards. Protocol numbers: OSHREC 16/4/2021/309 (Ministry of Health, Ondo State), ESUTHP/C-MAC/RA/034/VOL 3/120 (ESUT Teaching Hospital, Enugu), ADM/E22/A/VOL.VII/14831676 (University of Benin Teaching Hospital), CRS/MH/HREC/022/Vol. 1/216 (Ministry of Health, Cross River State), NHREC/09/23/2010B (Plateau State Specialist Hospital, Jos), NHREC/17/03/2030 (Ministry of Health, Kano state), MOH/ADM/SUB/1152/1/663 (Ministry of Health, Katsina State), HMB/OFF/215/VOL II/564 (Benue State Hospital Management Board) MOH/SEC3/1/60 (Ministry of Health Jigawa State), SHREC Approval No 116/2022 (Ministry of Health, Borno State). Informed consent was obtained from the parents or caregivers of all participants.

Additional information

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