

Prevalence and Risk Factors for *Cryptosporidium* Diarrhea among Children Aged Five Years and below in Selected Health Institutions in Abakaliki, South-East Nigeria

Onyinye Ifeyinwa Nkeiru Onyekachi^{1,2*}, Uduma Victor Uduma³, Emeka Donald Ogiji⁴, Kenekchukwu Emmanuel Onyekachi³, Nweke Chinedu Idakari², Nneka Marian Chika-Igenyi³, Chidiebere Brown Ene², Chinwe Ifeoma Joe-Akunne⁵, Chinedum Christabel Amagwu⁶, Shedrach Chikezie Emeribe⁷, Felix Osogu Edegbe⁸, Bolaji Abdulazeez Akanni⁹, Chibuike Sunday Ugwuocha¹⁰, Kingsley Achi¹¹, Adamma Gloria Olisa¹², Uzochukwu Chimdindu Ibe¹³, Chiedozie Kingsley Ojide^{1,2}

¹Department of Medical Microbiology, Ebonyi State University, Abakaliki, Nigeria

²Department of Medical Microbiology, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria

³Department of Internal Medicine, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria

⁴Department of Pharmacology and Therapeutics, University of Liverpool, United Kingdom

⁵Department of Pediatrics, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi Nigeria

⁶Department of Medical Microbiology, Enugu State University Teaching Hospital, Parklane, Nigeria

⁷Department of Medical Microbiology, University of Nigeria, Enugu Campus, Enugu, Nigeria

⁸Department of Anatomic Pathology, Ebonyi State, University, Abakaliki, Nigeria

⁹Department of Surgery, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria

¹⁰Department of Microbiology, Alex Ekwueme Federal University Ndufu-Alike Ikwo, Abakaliki, Nigeria

¹¹Department of Medical Laboratory Sciences, University of Nigeria, Nsukka, Nigeria

¹²Department of Public Health, Tennessee State University, Nashville, USA

¹³Department of Anatomic Pathology, Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria

Email: *onyinyeonyekachi91@gmail.com

How to cite this paper: Onyekachi, O.I.N., Uduma, V.U., Ogiji, E.D., Onyekachi, K.E., Idakari, N.C., Chika-Igenyi, N.M., Ene, C.B., Joe-Akunne, C.I., Amagwu, C.C., Emeribe, S.C., Edegbe, F.O., Akanni, B.A., Ugwuocha, C.S., Achi, K., Olisa, A.G., Ibe, U.C. and Ojide, C.K. (2025) Prevalence and Risk Factors for *Cryptosporidium* Diarrhea among Children Aged Five Years and below in Selected Health Institutions in Abakaliki, South-East Nigeria. *Advances in Microbiology*, 15, 1-18.

<https://doi.org/10.4236/aim.2025.151001>

Abstract

Background: Diarrheal diseases have globally decreased over the past few decades, yet they remain one of the top three causes of mortality in children under five years, especially in sub-Saharan Africa and Nigeria. Seasonal peaks of diarrheal episodes continue to contribute significantly to childhood mortality in these regions. One of the notable causes of diarrhea in children is parasitic infections, particularly *Cryptosporidium*, which poses a serious health risk. In Nigeria, the burden of *Cryptosporidium* diarrhea is under-researched, making it imperative to investigate its prevalence and associated risk factors. **Study Objectives:** The study aims to determine the prevalence and risk factors associated with *Cryptosporidium* diarrhea among children aged five years and

Received: November 20, 2024**Accepted:** January 4, 2025**Published:** January 7, 2025Copyright © 2025 by author(s) and
Scientific Research Publishing Inc.This work is licensed under the Creative
Commons Attribution International
License (CC BY 4.0).<http://creativecommons.org/licenses/by/4.0/>**Open Access**

below in selected health institutions in Abakaliki, South-East Nigeria. **Meth-
odology:** This cross-sectional study was conducted from January to May 2017,
recruiting 200 children under five years with diarrhea from health institutions
in Abakaliki. Fecal specimens were analyzed for *Cryptosporidium* oocysts us-
ing light microscopy with modified Ziehl-Neelsen staining and immunofluo-
rescent antibody test (IFAT). Deoxyribonucleic acid (DNA) was extracted
from positive samples using QIAmp® DNA stool kit, followed by Polymerase
Chain Reaction (PCR) and molecular genotyping. **Results:** *Cryptosporidium*
was detected in 0.5% (1/200) of children via light microscopy and 6.5%
(13/200) via IFAT. All positive samples were confirmed as *Cryptosporidium*
hominis by PCR. The prevalence of infection was significantly higher in chil-
dren from institutionalized homes (50.0%) compared to monogamous homes
(6.2%) ($p < 0.05$). Other associated risk factors included male gender, urban
residence, absence of exclusive breastfeeding, caregiver being a nanny, and
maternal lack of formal education. **Conclusion:** *Cryptosporidium hominis* is
a notable cause of diarrhea among children in Abakaliki, primarily transmit-
ted through human-to-human contact. The study underscores the need for
targeted interventions in childcare institutions to prevent outbreaks. Health
authorities should promote breastfeeding and enhance education on hygiene
practices in vulnerable populations.

Keywords

Cryptosporidium hominis, Immunofluorescent Antibody Test,
Ziehl-Neelsen Stain

1. Introduction

Diarrheal diseases remain a significant public health challenge, particularly in low- and middle-income countries, where children under the age of five are disproportionately affected [1]. Despite substantial global progress in reducing child mortality over the past few decades, the burden of diarrheal diseases continues to present a considerable challenge in certain regions, particularly in sub-Saharan Africa. Between 1990 and 2016, the global under-five mortality rate (U5MR) decreased from 93 deaths per 1000 live births to 41 per 1000, reflecting global advances in child health interventions [2]. However, in sub-Saharan Africa, the decline, though evident, has been less pronounced, with the U5MR falling from 183 to 79 deaths per 1000 live births over the same period [3]. Diarrheal diseases are not only a major cause of under-five mortality but also contribute significantly to malnutrition in these vulnerable populations [4]. In 2016, diarrheal diseases accounted for 8% of global under-five deaths, ranking as the fourth leading cause of child mortality, a burden that is most acute in sub-Saharan Africa and Southern Asia [3].

Nigeria, in particular, has emerged as a key focal point in this crisis, ranking second only to India in terms of the absolute number of under-five deaths

attributable to diarrheal diseases [3]. The country's elevated mortality rates can be attributed to a confluence of socio-economic and environmental factors, including limited access to clean water, inadequate sanitation, and substandard hygiene practices [5]. These structural deficits are compounded by host-related factors such as poor nutritional status, gender, and the duration of breastfeeding, which further heighten the vulnerability of young children to diarrheal diseases [1]. The complex interplay of these risk factors underscores the need for targeted interventions to mitigate the mortality and morbidity associated with diarrhea in Nigeria and other similar settings.

Among the numerous pathogens implicated in childhood diarrhea, enteroparasites, including *Cryptosporidium* species, *Entamoeba histolytica*, and *Giardia lamblia*, play a critical role [6]. Notably, *Cryptosporidium* has emerged as the second most important pathogen following rotavirus, contributing significantly to the global burden of diarrheal disease in children under four years of age [7]. The Global Enteric Multicenter Study (GEMS) highlighted that *Cryptosporidium* is responsible for approximately 2.9 million cases of moderate-to-severe diarrhea (MSD) in children aged 0 to 2 years in sub-Saharan Africa alone, leading to an estimated 202,000 deaths annually among children under 24 months in this region and South Asia [8]. Given the significant role *Cryptosporidium* plays in childhood morbidity and mortality, its surveillance is critical in understanding its epidemiology, particularly in resource-limited settings where the burden is highest.

Despite the known importance of *Cryptosporidium* in causing diarrhea among children, the epidemiological data on its prevalence and associated risk factors remain sparse and often outdated in many parts of Nigeria. Previous studies in the country have either focused on limited populations or have failed to comprehensively address the role of *Cryptosporidium* as a causative agent of diarrheal disease [9]-[11]. Moreover, there is a growing recognition of the need to explore specific regional variations in the prevalence of the parasite, particularly in areas like Abakaliki, South-East Nigeria, where local health policies may benefit from updated data on the pathogen's transmission dynamics.

The present study seeks to address this gap by investigating the prevalence of *Cryptosporidium* and identifying key risk factors for its transmission among children aged five years and below in selected health institutions in Abakaliki. Understanding the local epidemiology of *Cryptosporidium* is essential for guiding effective public health interventions and informing policy decisions aimed at reducing the burden of diarrheal diseases in Nigeria. This study is particularly timely, as it not only contributes to the global discourse on child health but also provides critical insights for local policymakers to formulate targeted interventions to curb the spread of *Cryptosporidium* infections.

2. Materials and Methods

2.1. Study Area

The study was conducted in the Pediatrics unit of Mile Four Hospital, and

Maternal and Child Health (MCH) Center, Abakaliki. Mile Four Hospital is a faith-based secondary level, not-for-profit hospital, located in Abakaliki, and well renowned for its management of tuberculosis and leprosy infections. It also has a very busy maternal and child health service, second only to Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFUTHA) in terms of patient attendance. MCH is a government-owned and managed primary health center located in the heart of the city. It runs health promotion and preventive services, which include antenatal care, delivery, and immunization services, as well as outpatient clinics for common illnesses and injuries. It is the busiest primary health center in the city.

2.2. Study Design and Population

The study was a hospital-based cross-sectional descriptive study carried out among children presenting at the Pediatrics units of Mile Four hospital and Maternal and Child Health (MCH) hospital. Inclusion criteria included children aged five (5) years and below, who presented with features of diarrheal diseases, and whose caregivers gave an informed consent. All children who met the inclusion criteria were recruited consecutively till the desired sample size was achieved.

2.3. Sample Size Determination

The minimum sample size was estimated using the formula

$$n = z^2 pq / d^2. \quad [12]$$

where n = desired population size, z = level of significance at 95% CI (=1.96), p = prevalence of *Cryptosporidium* positive fecal samples among under-fives in Ibadan, South-West, Nigeria (13.5%) [13], $q = 1 - p = 0.865$, and d = degree of accuracy desired, set at 0.05. We adjusted for 10% non-response rate. A minimum sample size of 200 was estimated.

2.4. Data Collection and Study Duration

Data collection was done using a semi-structured, interviewer-administered, pre-tested questionnaire, and was carried out by trained research assistants. The questionnaire included hospital identification number, and sections on biodata, family history, presenting complaint, feeding history, immunization history, treatment history, and patient outcome. The validity of the questionnaire was checked by having other members of the research team look through the questions to confirm that the questions asked passed their intended meaning. A one-month feasibility study was carried out in December 2016, and the main study (sample/data collection) was done over a five (5) month period, from January 2017 to May 2017.

2.5. Specimen Collection, Transport, and Storage

About 3 - 5 mls of fresh fecal samples were collected in dry, clean, leak-proof plastic bottles containing an equal amount of absolute methanol, and each labeled

according to the child's hospital identification number and hospital name.

Fecal samples were transported in absolute methanol to the laboratory within 24 hrs of collection and refrigerated at 4°C for microscopy. Collection and storage of samples were in line with the specifications of the US Center for Disease Control and Prevention [14].

Aliquots of each sample were put in dry, clean, leak-proof plastic bottles, each with an equal amount of absolute methanol, and shipped to a molecular laboratory in Oslo, Norway for serology and molecular analyses.

2.6. Laboratory Analysis

Macroscopy: Fecal samples were examined macroscopically for consistency, presence of blood, mucus, and presence of adult worms.

Microscopy: The modified cold acid-fast technique was used to stain the *Cryptosporidium* oocysts. Medium to thick smears of methanol-preserved stool were made on the slides and allowed to air dry, and then fixed in methanol for 3 minutes and air dried. The slides were then flooded with 3% Carbol Fuchsin for approximately 15 minutes and rinsed with tap water. The slides were also decolorized with 1% acid methanol, left for 15 - 20 seconds, rinsed with tap water, counter-stained with Methylene blue for 30 seconds, rinsed with tap water again, air-dried, and then examined using high and low dry objectives [15].

Serologic and Molecular: Serology and molecular analyses were done in the Parasittologisk Laboratorium, Institutt for Mattrygghet og Infeksjons biologi, NMBU, Oslo, Norway. Standard Immunofluorescent Antibody Test (IFAT) was done on all fecal samples to determine those positive for *Cryptosporidium*. 10µl of each sample was put on a slide, air-dried, fixed in methanol, and then stained with FITC-labelled monoclonal antibody (Mab: Aqua-glo, Waterborne Inc., New Orleans, USA) and 4', 6-diamidino 2 phenylindole (DAPI). The *Cryptosporidium* oocysts and DAPI inclusions were then noted [11].

From all the samples positive for *Cryptosporidium* oocysts, deoxyribonucleic acid (DNA) was extracted using the QIAmp® DNA Stool kit, following the manufacturer's protocol (QIAGEN, Hilden, Germany). Further molecular investigation, according to published primers and protocols, were carried out using fragments of SSU rRNA and GP60 genes, from samples positive for *Cryptosporidium* [11] [16] [17].

2.7. Data Analysis

Data obtained were analyzed using Epi-Info version 7.2 statistical software (CDC, Atlanta, Georgia, USA). Data were summarized using frequency tables. Associations between the dependent and independent variables were analyzed with Chi-square. Differences were considered significant at 5% level of significance.

2.8. Ethical Issues

Ethical approval was obtained from the Ebonyi State University Research Ethical

Committee, with reference EBSU/DRIC/UREC/Vol.02/026. The management of Mile Four Hospital and Maternal/Child Health Center, Abakaliki, also gave their approval for the study. Written informed consent was obtained from the care givers of the participating children after the procedure had been fully explained to them. Participation in the study was voluntary, and participants could withdraw their consent in the course of the study whenever they wished, without prejudice to the quality of treatment they received.

3. Results

3.1. Socio-Demographic Characteristics

122 (61.0%) of the 200 participants that took part in the study were males. The mean age of the males was 9.6 ± 4.8 months, while that of the females was 11.5 ± 8.5 months. 2 (1.0%) of the participants lived in institutional homes (Motherless babies' homes), while 3 (1.5%) and 195 (97.5%) lived in polygamous and monogamous home settings respectively. The mothers of 52 (26.0%) children had no formal education, while 9 (4.5%) of the mothers had a primary level of education as their highest qualification (**Table 1**).

Table 1. Sociodemographic characteristics of participants.

Parameter	Frequency (n)	Percentage (%)
Sex		
Male	122	61.0
Female	78	39.0
Age Group (months)		
0 - 11	137	68.5
12 - 24	56	28.0
25 - 60	7	3.5
Mean age (months)		
Male	9.6 [^]	4.8 [*]
Female	11.5 [^]	8.5 [*]
Family Type		
Monogamous	195	97.5
Polygamous	3	1.5
Institutional home	2	1.0
Highest Level of Education of Mother		
No formal education	52	26.0
Primary education	9	4.5
Secondary education	109	54.5
Tertiary education	30	15.0

Continued

Father's Occupation		
Professional	5	2.5
Top Businessman	62	31.0
Top Civil Servant	30	15.0
Politician	2	1.0
Middle level Business	9	4.5
Artisan	3	1.5
Unskilled	89	44.5
Caregiver		
Mother	197	98.5
Nanny	3	1.5
Place of Residence		
Rural	111	55.5
Urban	89	44.5

^mean age *Standard deviation of age.

3.2. Feeding and Environmental Hygiene Characteristics of Participants

56 (28.0%) out of the 200 participants were exclusively breastfed—18 (32.1%) for less than four months and 38 (67.9%) for greater than four months. The rest (144, 72.0%) were not exclusively breastfed (**Table 2**). Regarding the sources of drinking water, 154 (77.8%) took processed water, such as sachet water, bottled water, etc., 37 (18.75%) took borehole water, 3 (1.5%) took rain water, 2 (1.0%) got theirs from streams, and 2 (1.0%) got their drinking water from Wells (**Table 2**). For the toilet facilities used by the family, 128 (64.0%) out of 200 children used water closets, 33 (16.5%) used Pit toilets, and 39 (19.5%) used the open defecation system of sewage disposal (**Table 2**).

Table 2. Feeding and environmental hygiene characteristics of participants.

Parameter	Frequency (200)	Percentage (%)
Exclusive Breastfeeding		
Yes	56	28.0
No	144	72.0
Duration of Exclusive Breastfeeding (n = 56)		
Less than 4 months	18	32.1
4 - 6 months	38	67.9
Source of Drinking water		
Processed Water	154	77.8
Borehole	37	18.7

Continued

Rain water	3	1.5
Stream	2	1.0
Well	2	1.0
Family Toilet Type		
Water closet	128	64.0
Pit toilet	33	16.5
Open defecation	39	19.5

3.3. Prevalence of *Cryptosporidium* Diarrhea in Relation to Identified Demographic Risk Factors in Participants

Of the 200 fecal samples from the subjects screened for *Cryptosporidium* infection, 13 (6.5%) were positive by immunofluorescent antibody test (**Figure 1**).

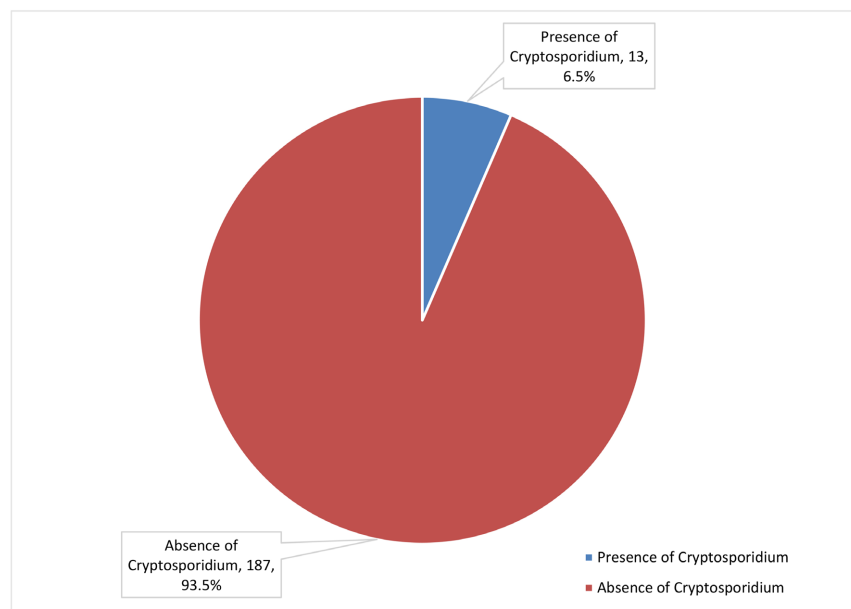


Figure 1. Prevalence of *Cryptosporidium* infection among subjects with diarrhea.

Cryptosporidium infection was most prevalent (50.0%; 1/2) among the children from institutional homes (motherless babies' homes) when compared to those from monogamous (6.2%; 12/195) and polygamous (0.0%; 0/3) homes, and this difference was statistically significant (**Table 3**). This indicates that children in institutionalized home settings were exposed to certain factors which put them at increased risk for *Cryptosporidium* diarrhea when compared to children in non-institutionalized home settings.

Cryptosporidium infection was also more prevalent in children whose mothers had no form of formal education (7.7%; 4/52) than in those whose mothers' highest educational level was the tertiary education (6.7%; 2/30) (**Table 3**). While there was no recorded case of *Cryptosporidium* infection among children whose

mothers' highest educational level was the primary education (0.0%; 0/9), the prevalence of the infection in diarrheic children of mothers with secondary education as their highest level of education was 6.4%; 7/109 (**Table 3**).

Table 3. Prevalence of *Cryptosporidium* infection in relation to identified demographic risk factors Participants.

Parameter	Number Screened (200)	Number Infected (13)	Percentage Positive (%)	Chi-square (p-value)
Sex				
Male	122	9	7.4	0.396 (0.529)
Female	78	4	5.1	
Place of Residence				
Rural	111	6	5.4	0.492 (0.483)
Urban	89	7	7.9	
Age Group (Months)				
0 - 11	137	8	5.8	1.129 (0.569)
12 - 24	56	5	8.9	
25 - 60	7	0	0.0	
Highest level of education of the mother				
No formal education	52	4	7.7	0.750 (0.861)
Primary education	9	0	0.0	
Secondary education	109	7	6.4	
Tertiary education	30	2	6.7	
Family type				
Monogamous	195	12	6.2	6.474 (0.039)
Polygamous	3	0	0.0	
Institutional home	2	1	50.0	
Father's Occupation				
Professional	5	0	0.0	4.327 (0.633)
Politician	2	0	0.0	
Top Civil servant	30	2	6.7	
Top businessmen	62	2	3.2	
Middle level businessman	9	0	0.0	
Artisan	3	0	0.0	
Unskilled worker	89	9	10.1	
Caregiver				
Mother	197	12	6.1	3.608 (0.057)
Nanny	3	1	33.3	

These differences in prevalence rates with reference to the highest educational levels of the mothers were however not statistically significant.

With reference to the occupation of their fathers, among the diarrheic children of the professionals, politicians, middle level businessmen, and artisans, there were no recorded cases of *Cryptosporidium* infection. The prevalence rates, however, among the children of the unskilled workers, top civil servants, and top businessmen were 10.1% (9/89), 6.7% (2/30), and 3.2% (2/62) respectively (**Table 3**). These differences were also not statistically significant.

The infection was more prevalent among the children whose primary caregivers were their nannies (33.3%; 1/3) than among those children whose primary caregivers were their mothers (6.1%; 12/197), but this too was not statistically significant (**Table 3**).

3.4. Prevalence of *Cryptosporidium* Diarrhea in Relation to the Identified Feeding and Environmental Hygiene Risk Factors

Of the 13 children positive with *Cryptosporidium* infection, 12 (8.3%; 12/144) were not exclusively breastfed while 1 was (1.8%; 1/56) (**Table 4**). This indicates a negative relationship between exclusive breastfeeding and *Cryptosporidium* infection. This finding was, however, not statistically significant.

Table 4. Prevalence of *Cryptosporidium* diarrhea in relation to the identified feeding and environmental hygiene risk factors in participants.

Parameter	Number Screened (200)	Cryptosporidium Positive (13)	Percentage Positive (%)	Chi Square (p-value)
Exclusive breastfeeding				
Yes	56	1	1.8	2.844 (0.092)
No	144	12	8.3	
Sources of drinking water				
Processed water	154	11	7.1	0.657 (0.957)
Borehole	37	2	5.4	
Rain water	3	0	0.0	
Stream	2	0	0.0	
Well	2	0	0.0	
Family toilet type				
Water Closet	128	9	7.0	0.191 (0.909)
Pit	33	2	6.1	
Open defecation	39	2	5.1	

While none of the participants positive for *Cryptosporidium* infection took rain water, stream water, or well water, 11 out of the 13 positive children (7.1%; 11/154)

had processed water as their source of drinking water, while 2 (5.4%; 2/37) had borehole as their source of drinking water (**Table 4**). This was statistically not significant too.

Out of the 13 children that tested positive for *Cryptosporidium species* in their diarrheal fecal samples, 9 used water closets (7.05; 9/128) as their means of sewage disposal, while 2 children each used pit latrines (6.1%; 2/33) and open defecation system (5.1%; 2/39) respectively (**Table 4**).

4. Discussion

The prevalence of *Cryptosporidium* infection observed in this study is lower than previous reports from Lagos in 2008, which focused on children under five years presenting with diarrhea [17]. However, it aligns more closely with findings from Kaduna State in 2014 [18]. This lower prevalence in Abakaliki is consistent with reports suggesting a general decline in diarrheal diseases over the years [2]. The 6.5% prevalence of *Cryptosporidium* infection found in this study is also lower than the 11.2% reported by Akinleye *et al.* in 2014 in Ibadan, where a broader age group of children aged 0 - 10 years was studied [19]. The restricted age range of children (0 - 5 years) in our study likely accounts for this difference.

Our study's prevalence of 6.5% contrasts with a 12.5% prevalence reported in a similar study conducted at Mulago Hospital in Kampala, Uganda, among diarrheic children aged 9 - 36 months [20]. The higher prevalence in Uganda may reflect the fact that *Cryptosporidium* infection peaks in otherwise healthy children between 6 - 12 months and is more common in immunocompromised or under-nourished children between 6 - 36 months [21]. These factors may not have been as prevalent in our study population, thereby explaining the lower prevalence in Abakaliki.

In this study, the only risk factor significantly associated with *Cryptosporidium* diarrhea was institutional home settings, where the prevalence of infection was markedly higher (50%) than in monogamous (6.2%) or polygamous (0.0%) households. This finding aligns with reports identifying *Cryptosporidium* as a significant pathogen in childcare institutions [22]. Given that all the detected species were *C. hominis*, a human-to-human transmitted parasite [23], it is plausible that once an outbreak occurs in an institutional setting, close contact and shared living conditions facilitate the spread of the infection among children.

The prevalence of *Cryptosporidium* infection was higher in urban areas (7.9%) compared to rural settings (5.4%), though this difference was not statistically significant. This pattern contrasts with a study in Ibadan, where the prevalence was higher in rural areas (11.8%) than urban areas (10.3%) [19]. This discrepancy may be explained by the fact that *C. hominis*, identified in our study, is typically associated with urban environments, where human-to-human transmission is more common [23]. In contrast, *Cryptosporidium parvum*, the species identified in the Ibadan study, is linked with zoonotic transmission, which is more prevalent in rural settings [24].

Our study also found that *Cryptosporidium* diarrhea was more common among children who were not exclusively breastfed (8.3%) compared to those who were (1.8%), although this difference was not statistically significant. This is consistent with previous studies, such as one conducted in 2012, which found that exclusive breastfeeding provides protection against diarrheal diseases [25]. Similarly, a 2014 study in rural and semi-rural areas of Tanzania identified that the onset of *Cryptosporidium* infection often coincided with changes in breastfeeding practices [26]. The small number of *Cryptosporidium*-positive cases in our study may have limited the statistical significance of this relationship, but the protective effect of exclusive breastfeeding remains evident [27].

Interestingly, our findings revealed a higher prevalence of *Cryptosporidium* infection (7.7%) among children whose mothers had no formal education, while those whose mothers had primary education recorded the lowest prevalence (0.0%). This finding is in line with a 2017 study that found children of uneducated mothers were more likely to suffer from diarrhea compared to those whose mothers had higher levels of education [28]. However, it contrasts with a study in Cotonou, Benin Republic, where the highest prevalence of cryptosporidiosis was observed among children of parents with only primary education [29]. This variation may stem from different breastfeeding practices, as higher levels of maternal education are often associated with lower rates of breastfeeding [30], which, as established, offers protection against diarrheal diseases [24] [31].

The highest prevalence of *Cryptosporidium* infection in our study occurred among children aged 12 - 24 months, a finding consistent with studies that show diarrhea becomes more common by six months of age [26]. However, this contrasts with findings from western Venezuela, where the highest prevalence was found in children younger than one year [32]. The explanation may lie in environmental factors, such as increased exposure to contaminated soil as children begin to crawl or walk, thereby increasing their risk of infection [32].

In terms of gender differences, the prevalence of *Cryptosporidium* infection was higher among males (7.4%) compared to females (5.1%), though this difference was not statistically significant. This contrasts with a study from Ibadan, where a higher prevalence was found in females [19], but aligns with findings from Sweden, where males had a higher prevalence of cryptosporidiosis [33]. The higher prevalence among males may be due to behavioral differences, with boys potentially being more likely to explore unsanitary environments [34]. However, most studies agree that gender is not a major risk factor for *Cryptosporidium* infection [33] [35].

Our study also found that children using water closets had a higher prevalence of infection (7.0%) compared to those using pit latrines (6.1%) or practicing open defecation (5.1%), though these differences were not significant. This contrasts with other studies that have found a significant association between open defecation and *Cryptosporidium* infection [32]. The use of non-sanitary toilet facilities may expose children to an increased risk of diarrheal diseases, including

Cryptosporidium infection [36].

This study has some limitations. The cross-sectional nature of the research only provides a snapshot of *Cryptosporidium* prevalence, without capturing seasonal or long-term trends. The sample size, while adequate, may still be insufficient to detect certain associations, especially with respect to exclusive breastfeeding and maternal education. Furthermore, the reliance on microscopy and molecular techniques limits the detection of other potential pathogens co-occurring with *Cryptosporidium*, which could have provided a more comprehensive picture of the diarrheal burden. These limitations suggest that further research with larger sample sizes and longitudinal designs is needed to fully understand the dynamics of *Cryptosporidium* infection in this region.

5. Conclusions

This study highlights a *Cryptosporidium* infection prevalence of 6.5% among children aged five years and below in Abakaliki, South-East Nigeria, with institutional home settings being the only significant risk factor. Other associated factors include male gender, lack of exclusive breastfeeding, and maternal education. These findings underscore the importance of targeted interventions to reduce the burden of *Cryptosporidium* diarrhea, particularly in childcare institutions, where human-to-human transmission appears to be a major contributor. The relatively higher prevalence in urban areas suggests that improving hygiene and sanitation, especially in densely populated regions, is crucial.

To address these findings, health policymakers should prioritize the improvement of sanitation facilities and hygiene practices, particularly in institutional childcare settings where overcrowding and close contact may facilitate the spread of infections. Healthcare providers are encouraged to intensify efforts to promote and support exclusive breastfeeding, which has been shown to offer protective benefits against diarrheal diseases. Additionally, community health workers should focus on educating mothers, particularly those with lower educational attainment, about the risks associated with poor hygiene and inadequate breastfeeding practices.

Public health authorities are urged to implement routine screening for *Cryptosporidium* in diarrheic children, particularly in institutional settings, to ensure early detection and effective management. Furthermore, additional research is recommended to explore seasonal trends and larger cohorts to enhance the understanding of *Cryptosporidium* infection dynamics in the region.

Acknowledgements

The authors would like to thank the administration of Parasittologisk Laboratorium, Institutt for Mattrygghet og Infeksjons biologi, NMBU, Oslo, Norway, for helping out in the serologic and molecular analyses.

Authors' Contributions

OINO and CKO conceptualized and wrote the draft of the article. OINO and KEO

anchored the sample and data collection. UVU anchored the data analyses and reviewed the manuscript. EDO, KEO, NCI, NMCI, CBE, CIJA, CCA, SCE, FOE, BAA, CSU, KA, AGO and UCI reviewed the manuscript.

Consent for Publication

The authors hereby transfer all copyright ownership exclusively to the journal, if this work is published by the journal.

Conflicts of Interest

The authors have declared no conflict of interest.

References

- [1] Bado, A.R., Susuman, A.S. and Nebie, E.I. (2016) Trends and Risk Factors for Childhood Diarrhea in Sub-Saharan Countries (1990-2013): Assessing the Neighborhood Inequalities. *Global Health Action*, **9**, Article ID: 30166.
<https://doi.org/10.3402/gha.v9.30166>
- [2] Troeger, C., Forouzanfar, M., Rao, P.C., Khalil, I., Brown, A., Reiner, R.C., *et al.* (2017) Estimates of Global, Regional, and National Morbidity, Mortality, and Aetiologies of Diarrhoeal Diseases: A Systematic Analysis for the Global Burden of Disease Study 2015. *The Lancet Infectious Diseases*, **17**, 909-948.
[https://doi.org/10.1016/s1473-3099\(17\)30276-1](https://doi.org/10.1016/s1473-3099(17)30276-1)
- [3] You, D., David, S., and Hug, L. (2017) Levels and Trends in Child Mortality: Report 2017 (English). Estimates Developed by the UN Interagency Group for Child Mortality Estimation.
<http://documents.worldbank.org/curated/en/358381508420391876/Levels-and-trends-in-child-mortality-report-2017>
- [4] Brown, K.H. (2003) Diarrhea and Malnutrition. *The Journal of Nutrition*, **133**, 328S-332S. <https://doi.org/10.1093/jn/133.1.328s>
- [5] WHO (2019) Partnership for Maternal, Newborn, and Child Health.
<https://www.who.int/nigeria/index1>
- [6] Tellevik, M.G., Moyo, S.J., Blomberg, B., Hjøllø, T., Maselle, S.Y., Langeland, N., *et al.* (2015) Prevalence of *Cryptosporidium Parvum/Hominis*, *Entamoeba histolytica* and *Giardia lamblia* among Young Children with and without Diarrhea in Dar Es Salaam, Tanzania. *PLOS Neglected Tropical Diseases*, **9**, e0004125.
<https://doi.org/10.1371/journal.pntd.0004125>
- [7] Murray, C.J., Ezzati, M., Flaxman, A.D., Lim, S., Lozano, R., Michaud, C., *et al.* (2012) GBD 2010: A Multi-Investigator Collaboration for Global Comparative Descriptive Epidemiology. *The Lancet*, **380**, 2055-2058.
[https://doi.org/10.1016/s0140-6736\(12\)62134-5](https://doi.org/10.1016/s0140-6736(12)62134-5)
- [8] Sow, S.O., Muhsen, K., Nasrin, D., Blackwelder, W.C., Wu, Y., Farag, T.H., *et al.* (2016) The Burden of *Cryptosporidium* Diarrheal Disease among Children < 24 Months of Age in Moderate/High Mortality Regions of Sub-Saharan Africa and South Asia, Utilizing Data from the Global Enteric Multicenter Study (GEMS). *PLOS Neglected Tropical Diseases*, **10**, e0004729.
<https://doi.org/10.1371/journal.pntd.0004729>
- [9] Okafor, J.I. and Okunji, P.O. (1994) Cryptosporidiosis in Patients with Diarrhea in Five Hospitals in Nigeria. *Journal of Communicable Diseases*, **26**, 75-81.
- [10] Ukwah, B.N., Ezeonu, I.M., Ezeonu, C.T., Roellig, D. and Xiao, L. (2017)

- Cryptosporidium Species and Subtypes in Diarrheal Children and HIV-Infected Persons in Ebonyi and Nsukka, Nigeria. *The Journal of Infection in Developing Countries*, **11**, 173-179. <https://doi.org/10.3855/jidc.8034>
- [11] Efunshile, A.M., Ezeanosike, O., Onyekachi, O.N.I., Ugwu, M.I., König, B. and Robertson, L.J. (2018) Apparent Absence of *Giardia* Infections among Children under 5-Years of Age with Acute Watery Diarrhoea in Abakaliki, Nigeria. *Epidemiology and Infection*, **147**, e58. <https://doi.org/10.1017/s0950268818003151>
 - [12] Araoye, M.O. (2004) Research Methodology with Statistics for Health and Social Sciences. Nathadex Publishers, 115-129.
 - [13] Centers for Disease Control and Prevention (2003) Diagnostic Procedures for Stool Specimens. <http://www.cdc.gov>
 - [14] Health Protection Agency (2007) Staining Procedures. National Standard Method Issue. http://www.hpa-standardmethods.org.uk/pdf_sop.asp
 - [15] Xiao, L., Escalante, L., Yang, C., Sulaiman, I., Escalante, A.A., Montali, R.J., *et al.* (1999) Phylogenetic Analysis of *Cryptosporidium* Parasites Based on the Small-Subunit rRNA Gene Locus. *Applied and Environmental Microbiology*, **65**, 1578-1583. <https://doi.org/10.1128/aem.65.4.1578-1583.1999>
 - [16] Sulaiman, I.M., Hira, P.R., Zhou, L., Al-Ali, F.M., Al-Shelahi, F.A., Shweiki, H.M., *et al.* (2005) Unique Endemicity of Cryptosporidiosis in Children in Kuwait. *Journal of Clinical Microbiology*, **43**, 2805-2809. <https://doi.org/10.1128/jcm.43.6.2805-2809.2005>
 - [17] Wellington, O., Chika, O., Teslim, O., Oladipo, O., Adetayo, F. and Godswill, I. (2008) Cryptosporidium and Other Intestinal Protozoans in Children with Diarrhea in Lagos, Nigeria. *The Internet Journal of Tropical Medicine*, vol. 5.
 - [18] Okojokwu, O.J., Inabo, H.E., Yakubu, S.E. and Okubanjo, O.O. (2014) Cryptosporidium Infection among Patients Presenting with Diarrhea in Kaduna State, Nigeria. *Researcher*, **6**, 36-42.
 - [19] Akinleye, O.M., Ogbolu, O.D., Fajolu, I.O., Oladejo, J.M., Adebisi, R.T. and Alo, O.G. (2014) Infection Rate of *Cryptosporidium parvum* among Diarrheal Children in Ibadan, Oyo State, Nigeria. *Scholars Journal of Applied Medical Sciences*, **2**, 3127-3131.
 - [20] Mor, S.M., Tumwine, J.K., Ndeezi, G., Srinivasan, M.G., Kaddu-Mulindwa, D.H., Tzipori, S., *et al.* (2010) Respiratory Cryptosporidiosis in HIV-Seronegative Children in Uganda: Potential for Respiratory Transmission. *Clinical Infectious Diseases*, **50**, 1366-1372. <https://doi.org/10.1086/652140>
 - [21] Mor, S.M. and Tzipori, S. (2008) Cryptosporidiosis in Children in Sub-Saharan Africa: A Lingering Challenge. *Clinical Infectious Diseases*, **47**, 915-921. <https://doi.org/10.1086/591539>
 - [22] Orenstein, W., Cordell, R.L. and Addiss, D.G. (1994) Cryptosporidiosis in Child Care Settings: A Review of the Literature and Recommendations for Prevention and Control. *The Pediatric Infectious Disease Journal*, **13**, 310-317. <https://doi.org/10.1097/00006454-199404000-00012>
 - [23] Chalmers, R.M., Smith, R., Elwin, K., Clifton-Hadley, F.A. and Giles, M. (2010) Epidemiology of Anthroponotic and Zoonotic Human Cryptosporidiosis in England and Wales, 2004-2006. *Epidemiology and Infection*, **139**, 700-712. <https://doi.org/10.1017/s0950268810001688>
 - [24] Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M., *et al.* (2013) Zoonotic *Cryptosporidium* Species and *Enterocytozoon bienersi* Genotypes in HIV-Positive Patients on Antiretroviral Therapy. *Journal of Clinical Microbiology*, **51**, 557-563. <https://doi.org/10.1128/jcm.02758-12>

- [25] Yilgwan, C. and Okolo, S. (2012) Prevalence of Diarrhea Disease and Risk Factors in Jos University Teaching Hospital, Nigeria. *Annals of African Medicine*, **11**, 217-221. <https://doi.org/10.4103/1596-3519.102852>
- [26] Pedersen, S.H., Wilkinson, A.L., Andreasen, A., Warhurst, D.C., Kinung'hi, S.M., Urassa, M., *et al.* (2014) Cryptosporidium Prevalence and Risk Factors among Mothers and Infants 0 to 6 Months in Rural and Semi-Rural Northwest Tanzania: A Prospective Cohort Study. *PLOS Neglected Tropical Diseases*, **8**, e3072. <https://doi.org/10.1371/journal.pntd.0003072>
- [27] Karambu, S., Matiru, V., Kiptoo, M. and Oundo, J. (2013) Characterization and Factors Associated with Diarrhoeal Diseases Caused by Enteric Bacterial Pathogens among Children Aged Five Years and Below Attending Igembe District Hospital, Kenya. *Pan African Medical Journal*, **16**, Article 37. <https://doi.org/10.11604/pamj.2013.16.37.2947>
- [28] Adeyimika T, D., Mojisola M, O., Yetunde O, J., Opeyemi, O. and S. Ayo, A. (2017) Maternal Education and Diarrhea among Children Aged 0-24 Months in Nigeria. *African Journal of Reproductive Health*, **21**, 27-36. <https://doi.org/10.29063/ajrh2017/v21i3.2>
- [29] Ogouyèmi-Hounto, A., Alihonou, F., Aholoukpe, I., Bagnan, L., Orekan, J., Sossa, B., *et al.* (2017) Prevalence of Cryptosporidiosis Infection among Children under 5-Years in Cotonou, Benin. *Journal of Parasitology and Vector Biology*, **9**, 89-94.
- [30] Zhao, J., Zhao, Y., Du, M., Binns, C.W. and Lee, A.H. (2017) Maternal Education and Breastfeeding Practices in China: A Systematic Review and Meta-analysis. *Midwifery*, **50**, 62-71. <https://doi.org/10.1016/j.midw.2017.03.011>
- [31] Bouzid, M., Kintz, E. and Hunter, P.R. (2018) Risk Factors for Cryptosporidium Infection in Low and Middle Income Countries: A Systematic Review and Meta-analysis. *PLOS Neglected Tropical Diseases*, **12**, e0006553. <https://doi.org/10.1371/journal.pntd.0006553>
- [32] Chacín-Bonilla, L., Barrios, F. and Sanchez, Y. (2008) Environmental Risk Factors for Cryptosporidium Infection in an Island from Western Venezuela. *Memórias do Instituto Oswaldo Cruz*, **103**, 45-49. <https://doi.org/10.1590/s0074-02762008005000007>
- [33] Adler, S., Widerström, M., Lindh, J. and Lilja, M. (2017) Symptoms and Risk Factors of Cryptosporidium Hominis Infection in Children: Data from a Large Waterborne Outbreak in Sweden. *Parasitology Research*, **116**, 2613-2618. <https://doi.org/10.1007/s00436-017-5558-z>
- [34] Siziya, S., Muula, A. and Rudatsikira, E. (2013) Correlates of Diarrhoea among Children Below the Age of 5 Years in Sudan. *African Health Sciences*, **13**, 376-383. <https://doi.org/10.4314/ahs.v13i2.26>
- [35] El-Badry, A.A., Al-Antably, A.S.A., Hassan, M.A., Hanafy, N.A. and Abu-Sarea, E.Y. (2015) Molecular Seasonal, Age and Gender Distributions of Cryptosporidium in Diarrhoeic Egyptians: Distinct Endemicity. *European Journal of Clinical Microbiology & Infectious Diseases*, **34**, 2447-2453. <https://doi.org/10.1007/s10096-015-2502-y>
- [36] Chowdhury, F., Khan, I.A., Patel, S., Siddiq, A.U., Saha, N.C., Khan, A.I., *et al.* (2015) Diarrheal Illness and Healthcare Seeking Behavior among a Population at High Risk for Diarrhea in Dhaka, Bangladesh. *PLOS ONE*, **10**, e0130105. <https://doi.org/10.1371/journal.pone.0130105>

Appendix

Questionnaire on the Prevalence and Risk Factors for *Cryptosporidium* Diarrhea Among Children Aged 5 Years and Below.

SECTION 1: HOSPITAL ID

- 1) Serial Number: -----
- 2) Hospital Name: -----
- 3) Hospital Number: -----
- 4) Month/Year of Admission: -----

Section 2: Biodata

- 1) Age of Child: Years ----- Months -----
- 2) Sex of Child: A. Male B. Female
- 3) Weight of Child: -----(kg)
- 4) Height of Child: -----(cm)

Section 3: Family History

- 1) Care Giver: A. Mother B. Nanny C. Granny D. Siblings E. Others (Specify)
- 2) Place of Residence: A. Rural B. Urban
- 3) Family type: A. Monogamous B. Polygamous C. Others (Specify -----)
- 4) Mother's Highest Education: A. Postgraduate B. First Degree C. SSCE D. FSLC E. None
- 5) Father's Occupation: A. Professional B. Top Civil Servant C. Politician D. Middle level Bureaucrats E. Top Business men F. Skilled Artisans G. Unskilled Worker

Section 4: Presenting Complaint

- 1) When did the diarrhea start? (DD/MM/YY) -----
- 2) When did it end? (DD/MM/YY) -----
- 3) Any vomiting? A. Yes B. No
- 4) Any Fever? A. Yes B. No
- 5) Any Blood in Stool? A. Yes B. No
- 6) Any mucus in Stool? A. Yes B. No

Section 5: Feeding History

- 1) Exclusively breast fed? A. Yes B. No
- 2) If (1) above is Yes, how long did it last A. <4 months B. 4 - 6 months
- 3) Current form of feeding A. Exclusive Breastfeeding B. Complementary feeding C. Predominant feeding D. Exclusive Formula feed E. Supplementary/Mixed feeding
- 4) If Complementary, what are the constituents?
 - A) Commercial Cereals, e.g., Cerelac®
 - B) Local feeds, e.g., Pap

- 5) If Pap, fortified with -----
- 6) Is the baby on bottle feeding? A. Yes B. No
- 7) Who prepares the baby's food? A. Mother B. Nanny C. Granny D. Older Siblings E. Others (Specify -----)
- 8) What is the Child's source of Drinking Water? A. Vendors (e.g., Tankers) B. Borehole C. Well D. Rain E. Processed (e.g., Sachet, Bottle) F. Stream
- 9) Any form of Water treatment? A. None B. Boiling C. Chemical (Chlorine) D. Filtering

Section 6: Immunization History

- 1) Did the Child receive Rotavirus vaccine? A. Yes B. No
- 2) If YES where? A. AEFUTHA B. MILE 4 C. HEALTH CENTER D. PRIVATE HOSPITAL (Specify -----)
- 3) Did the Child receive Measles vaccine? A. Yes B. No

Section 7: Treatment History

- 1) Medications given to the Child:
- | | | |
|--------------------------------------|--------|-------|
| ➤ IVF | A. Yes | B. No |
| ➤ ORS | A. Yes | B. No |
| ➤ VIT A | A. Yes | B. No |
| ➤ ZINC | A. Yes | B. No |
| ➤ PROBIOTICS e.g., Floranorm | A. Yes | B. No |
| ➤ ANTIBIOTICS | A. Yes | B. No |
| ➤ ANTI-DIARRHEALS e.g., Diastop | A. Yes | B. No |
| 2) Feeding during illness | A. Yes | B. No |
| 3) If YES specify type of Food ----- | | |

Section 8: Outcome/Follow-Up

Phone Number of Care-Giver.....