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**Research Article**

# Prevalence, Subtypes and Transmission Potential of *Cryptosporidium* in Humans in Federal Capital Territory, Nigeria

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**Abstract**
*Cryptosporidium* is a leading cause of diarrhoeal diseases in children and immuno-compromised individuals and transmissible from livestock to humans. The risk factors, subtype families, and transmission potential of *Cryptosporidium* in humans were examined in the Federal Capital Territory, Nigeria from January to September 2022. A total of 376 stools discriminated based on sex, age of the patients, nature of faeces, consumption of raw vegetables and contact with animals were analysed using PCR protocol. The PCR yielded 4 positive isolates. Prevalence was higher in males, those practicing open defecation, consumers of raw vegetables and those in contact with animals while it was highest in infants and Type 6 BSFS group. The study suggested consumption of vegetables (P = 0.001) and close contact with animals (P = 0.0356) as likely risk factors of the pathogen. The RFLP and partial sequencing of the 60-kDa glycoprotein (gp60) gene conducted on the 4 PCR positive isolates respectively yielded *Cryptosporidium parvum* (n = 1; 25%) and *C. hominis* (n = 3; 75%). Further analysis revealed the isolates to be subtype family *C. parvum* IIc and subtype family *C. hominis* Ia. The *C. hominis* Ia subtype family were of 2 subtypes. They were subtype IaA25R3 with a higher frequency of occurrence (66.7%) and subtype IaA23R3 (33.3%). The *C. parvum* subtype family detected was subtype IIcA5G3k (100%). The mix of *C. parvum* and *C. hominis* species and subtype families detected in this study suggests a likelihood of anthroponotic transmission of cryptosporidiosis in humans in Federal Capital Territory, Nigeria.
**Keywords:** *Cryptosporidium*, Humans, Open Defecation, Anthroponotic, Subtype-Family, Zoonotic.

## 1. Introduction

*Cryptosporidium* species is an intracellular protozoan parasite causing morbidity, mortality and economic losses by their effect on the gastrointestinal tract of vertebrate animals and humans (Vanathy *et al.,* 2017). It is one of the diseases linked to environmental contamination from wastes from humans and livestock. Contamination of cattle manure has reportedly led to many foodborne and waterborne outbreaks of human cryptosporidiosis (Blackburn *et al.,* 2006; Glaberman *et al.,* 2002). Nigeria is an agrarian society where livestock are wantonly raised especially within areas of human dwellings with little or no regulations. Cryptosporidiosis has become the leading cause of pediatric morbidity and mortality (Robertson *et al.,* 2020) consequent upon the close relationship of humans and animals through zoonotic exposure (Kotloff *et al.,* 2013; Snelling *et al.,* 2007). *Cryptosporidium* has been described as the commonest pathogen in patients with HIV, immuno-defiency and diarrhoea (Gerace *et al.,* 2019).

*Cryptosporidium* is also the most frequently isolated pathogen in HIV-negative children under 2 years of age with acute diarrhoea (Korpe *et al.,* 2018). Diarrhea is rated as the second biggest killer of children in Nigeria and estimated to kill 151,700 children every year (Dairo *et al.,* 2017). Robertson *et al.,* (2020) observed that works on characterization of *Cryptosporidium* into species, genotypes and sub-genotypes using molecular devices in many parts of Africa are very few due to deficit in infrastructure for conducting the molecular protocols. Federal Capital Territory is described as having the fastest annual population growth of 7.1% in Nigeria, high infrastructural deficit, rampant water shortages and open defecation and poor sanitation is a destination for job seekers, business investors and government officers. It is replete with unregulated restaurants, open fruit and vegetable markets, wanton rearing of cattle in human settlements. Against this

background and the rising spate of diarrhea in Nigeria, this study aimed at employing molecular tools at investigating the prevalence, characteristics and zoonotic potential of *Cryptosporidium* in humans in Federal Capital Territory, Nigeria.

## 2. Materials and Methods

### 2.1. Institutional Review Board Statement

"All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Health Research Ethics Committee of the Federal Capital Territory with Ethics number: FHREC/2022/01/210/04-11". Informed consent was obtained from each participant whose stool was collected and answered to our questionnaire. Participation by those enlisted in the study was voluntary after an explanation to them on the objectives of the research. Consent of the infants and children in this study was given on their behalf by their parents and guardians after a detailed explanation to them of the object of the research, what is expected of the participants, risks and benefits of the research and the fact that participation is voluntary. Medical record data of the participants were obtained after the permission of the superintending health authorities and the consent of the patient was obtained.

### 2.2. Inclusion Criteria

Included in this study were infants, toddlers, children and adult patients up to seventy years with or without diarrhea, clinically malnourished children, diabetic patients, HIV and tuberculosis patients and pregnant women.

### 2.3. Questionnaire

The questionnaire was prepared in English language and translated into the three major languages spoken in the study area (Hausa, Yoruba and Gwari) to enhance communication with participants with no formal education. The content, relevance, understanding, design and simplicity of the questionnaire were assessed and reviewed by subject experts. The questionnaire was administered to the consenting patients with the help of laboratory scientists alongside an interpreter to collect data on potential risk factors of *Cryptosporidium* infection. Patients' age were classified based on Manufacturer Help Center (2017) age groupings as infants (3-12 months old), toddlers (1-5 years), children (5-13 years) and adults (13 and above), sex (males and females), nature of stool based on types (Chira and Dumitrascu, 2015). Consumption of raw vegetables (described as eating raw vegetables grown in farms fertilized from cattle dungs), contact with animals (defined as livestock herder living within cattle farm premises) and defecation facilities classified as open defecation (defined as open space having no facility) and those with toilet facilities (flush toilet to piped sewer system and septic tank). The completed questionnaires were all collected, verified for accuracy, and thereafter labelled and coded.

### 2.4. The Study Area

The study area, Federal Capital Territory (FCT), Nigeria consists of Abaji, Abuja Municipal (AMAC) Bwari, Gwagwalada, Kwali and Kuje area councils. With a population of 2,238,800 (NPC, 2006), FCT covers a landmass of 7,315km$^2$ with a human density of 306/km$^2$. The FCT (Latitude: 8°25´ and 9°20´N of the Equator; Longitude 6°45´ and 7°39´E of Greenwich Meridian) is populated mostly by Gbagyl indigenous tribe whose major occupation is farming (Wikipedia, 2017). The territory has hot dry season and a short period of harmattan in between these seasons with an annual total rainfall of 1100 and 1600mm from April to October and lies in the Guinean forest-savannah mosaic zone of the West African sub-region (Abubakar, 2014).

### 2.5. Sampling and Transportation of Specimen

The sampling was done in the outpatient wards of the two main federal government owned referral/tertiary hospitals in the FCT comprising University of Abuja Teaching Hospital (UATH), Gwagwalada and National Hospital (NH), Garki, between January and September 2022. Single fresh stool samples were taken from each of the 200 enlisted patients at each of the hospitals. About 1g of stool out of the samples brought for analysis at the parasitology laboratory of each of the hospitals was gently discounted with the help of a spatula in a sterile bottle containing 2.5% (w/v) potassium dichromate and made into an 8 mL aliquot. The aliquot was thoroughly mixed and transported in cold ice packs to DNA Laboratory, Ali Akilu Road, Kaduna, Nigeria for characterization. Samples were kept at 4°C before DNA isolation.

### 2.6. DNA Extraction and *Cryptosporidium* Detection and Typing

Centrifugation (1500g for 10 min) was conducted on the stool specimen to wash off the potassium dichromate preservative. The DNA of the specimen were then extracted using FastDNA SPIN Kit for soil (BIO

101, Carlsbad, CA). The DNA preparations obtained were subjected to Polymerase Chain Reaction (PCR) analysis using the small subunit rRNA gene of the parasite. Subsequently, Restriction Fragment Length Polymorphism (RFLP) technique was used in detecting and differentiating the isolates into genotypes (Cama *et al.,* 2007) in the human samples. The genotypes were further subtyped using PCR-sequencing tool that targets the 850-bp fragment of the 60 kDa glycoprotein (gp60) gene of the isolates (Cama *et al.,* 2007).

### 2.7. DNA Sequence Analysis

The secondary PCR products of gp60 were purified using the Microcon PCR Centrifugal Filter Devices (Millipore, Bedford, MA) and sequenced directly, using an ABI BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) and ABI 3130 genetic analyzer (Applied Biosystems). The sequences obtained were read using the software ChromasPro (www.technelysium.com.au/ChromasPro.html), edited manually using the BioEdit program version 7.0.4 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), and aligned with reference sequences of each target using the GenRunner software (v. 3.05). Subtypes nomenclature was done according to the number of trinucleotide repeats (TCA or TCG) coding for the amino acid serine (Sulaiman *et al.,* 2005).

## 3. Results

### 3.1. Prevalence and Risk Factors

An overall prevalence of 1.1% (4/376) *Cryptosporidium* in humans in the FCT, Nigeria using PCR detection protocols was obtained. In this study, prevalence of *Cryptosporidium* was not associated with age (P = 0.792) and nature of stool (P = 0.293) although prevalence was highest in the infants, 1.4% (2/146) among other groups and BSFS Type 6 stools, 4.4% (2/45) highest among other stool forms.

Prevalence of *Cryptosporidium* was higher in patients that practiced open defecation 1.7% (3/174) compared to those that had access to modern toilet facilities 0.5% (1/202) while infection in male participants was higher 2.0% (3/151) compared to females, 0.4% (1/225). There appears to be an association of prevalence of *Cryptosporidium* in patients that had contact with animals 6.5% (2/31) compared to 1.6% (2/126) in those that had no contact (P = 0.030). An association (P = 0.001) was also demonstrated in the prevalence of *Cryptosporidium* in those that consumed raw vegetables 4.3% (4/94) compared to the zero infection of *Cryptosporidium* recorded in those that did not (Table 1).

**Table 1**. Showing potential risk factors of *Cryptosporidium* infection in humans in FCT.

| Variables | No. sampled | No. positive (%) | Chi-square | P-value |
|---|---|---|---|---|
| National hospital | 184 | 1 (0.5) | 0.93 | 0.336 |
| UATH | 192 | 3 (1.6) | | |
| **Sex** | | | | |
| Male | 151 | 3 (2.0) | 0.74 | 0.062 |
| Female | 225 | 1 (0.4) | | |
| **Age** | | | | |
| 3-12 (infants) | 146 | 2 (1.4) | 1.04 | 0.792 |
| 1-5 (toddlers) | 48 | 0 (0) | | |
| 5-13 (children) | 109 | 1 (0.9) | | |
| 13-65 (adults) | 73 | 1 (0.1) | | |
| **Nature of feces (BSFS) on** | | | | |
| Type 6 | 45 | 2 (4.4) | 3.72 | 0.293 |
| Type 7 | 87 | 1 (1.1) | | |
| Type 5 | 53 | 1 (2.0) | | |
| Type 4 | 191 | 0 (0) | | |
| **Vegetables** | | | | |
| Yes | 94 | 4 (4.3) | 12.13 | 0.001 |
| No | 282 | 0 (0) | | |
| **Defecation** | | | | |
| Open defecation | 174 | 3 (1.7) | 1.34 | 0.246 |
| Toilet facilities | 202 | 1 (0.5) | | |
| **Contact with animals** | | | | |
| No | 345 | 2 (0.6) | 4.25 | 0.0356 |
| Yes | 31 | 2 (6.5) | | |
| *No = number, *UATH = University of Abuja Teaching Hospital | | | | |

### 3.2. Genotypes and Subtypes

Digestion of the PCR products using Restriction Fragment Length Polymorphism (RFLP) technique revealed them as *Cryptosporidium parvum* and *C. hominis*. Sequence analysis of the gp60 gene from the DNA of the isolates revealed that the single *C. parvum* isolate belonged to subtype family IIc (1/4; 25%), while all three *C. hominis* isolates (3/4; 75%) were classified within subtype family Ia. The *C. parvum* subtype family IIc was 100% (1/1) subtype IIcA5G3k while the *C. hominis* subtype family Ia was subtype 33.3% (1/3) IaA23R3 (33.3%) and subtype 66.6% (1/3) IaA25R3. The pattern of distribution of the isolates is as presented in Table 2.

**Table 2.** Characterization of *Cryptosporidium parvum* and *Cryptosporidium hominis* subtype families in humans in FCT, Nigeria.

| Genotypes | Number of isolates | Subtype families | Subtypes | Accession number |
|---|---|---|---|---|
| *C. parvum* | 1 | IIc | IIcA5G3k | YaK EF6133 |
| *C. hominis* | 3 | Ia | IaA23R3<br>IaA25R3 | AF16450 |

### 4. Discussion

The prevalence of *Cryptosporidium* in humans in FCT using 18s ss rRNA-PCR was 1.1%. This differs from the "no *Cryptosporidium* infection" findings of Banwat *et al.,* (2004) and Nwokediuko *et al.,* (2002) in Central and South-eastern, Nigeria respectively. The prevalence in this study is however below the 5-10% (Checkley *et al.,* 2015) described for poor countries and the 9–52.7% prevalence range in Nigeria earlier reported by Adeiza *et al.,* (2023). Differences in prevalence in these studies may be due to differences in detection methods, sample population and the study location (Adesiji *et al.,* 2007).

Prevalence of *Cryptosporidium* in this study was not be associated with age (P=0.7922) but skewed in favor of the infant age. The vulnerabilities of the infants to *Cryptosporidium* infection may be due to certain immunological incompetence often associated with the age-group (Hunter *et al.,* 2004; Roy *et al.,* 2004). This study revealed that prevalence of *Cryptosporidium* in males was higher than females. This result is similar to the reports of Egberongbe *et al*., (2010) and Dash *et al.,* (2017) but differs from Dozie *et al.,* (2017) who reported higher prevalence in females in Imo State, Nigeria. The traditional practice of men eating in commercial food houses may be responsible for the undue exposure of the males to *Cryptosporidium* infections in this study.

Prevalence of *Cryptosporidium* in participants that had contact with animals was higher in humans compared to those without contact. There was a significant association between prevalence of *Cryptosporidium* and contact with companion animals (P = 0.0356). This result concurs with the earlier studies of Sarkar *et al.,* (2014) and Maikai *et al.,* (2012) where contact with animals was reported to be associated with increased risk of cryptosporidiosis but differs from the report of Khan *et al*., (2019) where contact with animals was reported to be protective to *Cryptosporidium* infections. The differences may be due to the likelihood of infection from other sources other than contact with an infected animal (Fayer *et al*., 2000).

In this study, prevalence of *Cryptosporidium* revealed no association with the nature of stool (P = 0.29). The high prevalence of *Cryptosporidium* in patients with Bristol Stool Form Scale (BSFS) Type 6 stools in this study is however in tandem with the findings of Ogendo *et al*., (2017) who observed *Cryptosporidium* infection to be associated with the presence of mushy loose stool. This finding however contrasts the report of Siwila *et al.*, (2007) that immune-compromised humans with Type 7 (diarrheic) stool are at an increasing danger of disseminating zoonotic *C. parvum* isolates.

*Cryptosporidium,* according to Santin *et al*., (2008) may not be the sole cause of diarrhoea stools hence *Cryptosporidium* may be a facultative or incidental parasite. Four of the 94 patients (4.3%) in this study who consumed raw vegetables had significant *Cryptosporidium* parasites (P = 0.001) compared to the zero detection of *Cryptosporidium* in the group that did not consume. This finding tallies with the reports of Xiao and Ryan (2008) and Adeiza *et al.*, (2023) where *Cryptosporidium* infections was associated with consumption of raw vegetable man especially when eaten unwashed.

In this study, open defecation did not show association with prevalence of *Cryptosporidium* although, respondents indulging in the practice had higher prevalence of *Cryptosporidium*, 1.7% compared to 0.5% in those that had better toilet facilities. This result negates the assertion that, open defecation and poor sanitation contribute significantly to the risk of cryptosporidiosis (WHO/UNICEF Joint Water Supply, and

Sanitation Monitoring Programme, 2015). Two *Cryptosporidium* species, *C. hominis* (75%) which dominated *C. parvum* (25%) were detected in this study. The detection of these two species correlates previous report that cryptosporidiosis was mainly caused by *C. parvum* and *C. hominis* (Morgan-Ryan *et al*., 2002).

The dominance of *C. hominis* in this study further agrees with the study of Jex *et al*., (2008) that suggested the genotype as the most predominant in humans in developing countries. This pattern of dominance especially in children or human immunodeficiency virus-positive adults was previously reported in Peru, Thailand, Malawi, Uganda, Kenya, and South Africa (Gatei *et al*., 2003; Peng *et al*., 2003). Studies of Sulaiman *et al*., (2005) and Alves *et al.,* (2003) however reported dominance of *C. parvum* over other genotypes. The difference in distribution may be due to the probable differences in infection sources (Learmonth *et al*., 2004). Dominance of *C. hominis* may be an indication of less zoonotic transmission.

In two different studies, Xiao and Ryan (2004) and Xiao (2010) both independently reported cryptosporidiosis in humans to be caused by *C. parvum* subtype families IIa and IIc. In our study, we detected only *C. parvum* subtype family IIc subtype IIcA5G3k but further detected *C. hominis* subtype family Ia subtypes IaA25R3 and IaA23R3. The detection of *Cryptosporidium parvum* subtype family IIc in this study supports the report of King *et al.,* (2019) that *C. parvum* subtype IIc is the commonest in low-income countries with poor sanitation. It was isolated from a 4-months-old male infant with BSFS Type 7 stool.

The two *Cryptosporidium hominis* subtype IaA25R3 which predominated the other *C. hominis* subtype in this study were isolated from a 53 year-old HIV adult female with BSFS Type 6 stool and 6-months old male infant with BSFS Type 7 stool while the third *C. hominis* subtype, IaA23R3, was isolated from a malnourished 10-month-old male infant. *Cryptosporidium* subtype IIc had previously been reported in studies on diarrheal children and HIV patients in southern Nigerian states of Edo, Ebonyi and Oyo in Nigeria (Ayinmode *et al.,* 2014; Akinbo *et al.,* 2010).

The detection in this study of *C. hominis* subtype Ia and *C. parvum* IIc, reported to be majorly anthroponotic (Wang *et al.,* 2014), underscores a likelihood of mainly anthroponotic transmission of *Cryptosporidium* taking place in humans in FCT. This is consistent with the reports of earlier epidemiological studies in sub-saharan Africa that hypothesized a predominantly anthroponotic transmission in the region (Squire and Ryan, 2017; Mbae *et al.,* 2015) and the report of Xiao (2010) asserting that largely human-specific *C. hominis* and zoonotic *C. parvum* subtype family IIc are most often responsible for the epidemiology of human cryptosporidiosis (Xaio, 2010).

## 5. Conclusion

This study suggests consumption of raw vegetables and having contact with animals as likely risk factors of spread of cryptosporidiosis in humans in FCT, Nigeria. The detection of *C. parvum* subtype family IIc and *C. hominis* subtype family Ia which are both anthroponotic suggest anthroponotic rather than zoonotic transmission of *Cryptosporidium* takes place in humans in FCT, Nigeria. We were limited in this conclusion by the paltry positive samples obtained in this study, inadequate fund for a more comprehensive survey involving larger population and a more sophisticated molecular detection tool.

## Declarations

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**Author Contributions:** The authors confirm sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

**Conflict of Interest:** The authors declare no conflict of interest.

**Consent to Publish:** The authors agree to publish the paper in International Journal of Recent Innovations in Academic Research.

**Data Availability Statement:** The data presented in this study are available upon request from the corresponding author.

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**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Health Research Ethics Committee of the Federal Capital Territory with Ethics number: FHREC/2022/01/210/04-11.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in this study.

**Research Content:** The research content of manuscript is original and has not been published elsewhere.

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