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Prevalence of *Cryptosporidium* Infection among Children Attending Maryam Abacha Women and Children Hospital, Sokoto State, Nigeria

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Abstract

Cryptosporidium infection is ranked as the second most common cause of diarrheal disease and fatalities among children in developing nations. Nonetheless, due to the fact that many healthcare providers do not consistently request Cryptosporidium diagnostic tests, the prevalence of cryptosporidiosis is probably underestimated. The current study is therefore aimed at investigating the prevalence of cryptosporidiosis among children attending Maryam Abacha Women and Children Hospital (MAWCH), Sokoto. During the timeframe of April to June 2021, stool samples were collected from 114 diarrheic and non-diarrheic children and analyzed using Formol ether concentration and Modified Ziehl Neelsen staining methods. Out of 114 samples analyzed, 81(71.1%) were positive for Cryptosporidium oocysts. A higher prevalence of Cryptosporidiosis was observed in males 45(39.5%) compared to females 36(31.6%) participants and higher 41(36.0%) in younger children aged 0-1 years than the older children. Similarly, a greater prevalence of Cryptosporidiosis was observed in diarrheic children (42.1%) than in non-diarrheic children (28.9%). A statistically significant relationship was observed between the occurrence of Cryptosporidium and diarrhea (P = 0.002) and the source of drinking water (P = 0.001). Routine diagnosis of Cryptosporidiosis among children is vital and can enhance effective diagnosis, treatment, and consequently, improvement in the patients' well-being.

Keywords: Cryptosporidiosis, Diarrhea, Children, Modified ZN staining

INTRODUCTION

Cryptosporidiosis is a disease caused by infection with *Cryptosporidium*, a type of coccidian protozoan parasite. This condition typically leads to watery diarrhea that can be severe and long-lasting. *Cryptosporidium* can infect the gastrointestinal tract of various vertebrates, such as humans, domestic and wild animals, and birds (Fayer *et al.*, 2008). Cryptosporidiosis was initially identified in mice in 1912, and its connection to human diseases was established in 1976 (WHO, 2004). In humans, almost 20 different species of *Cryptosporidium* have been identified, including *C. hominis* (formerly known as the *C. parvum* human genotype), *C. parvum* (bovine genotype), *C. meleagridis, C. canis*, and *C. felix*, which are the main culprits for human infections. *C. hominis* is a genotype that affects only humans, while *C. parvum* (zoonotic genotype) can infect both humans and a variety of domestic and wild animals, being the most commonly reported species (Xiao and Feng, 2008).

Cryptosporidium is transmitted through the fecal-oral route, either through direct or indirect contact with *Cryptosporidium* oocysts. This can occur through person-to-person contact, zoonotic transmission, waterborne exposure, foodborne contamination, or even airborne contact. The oocysts of these parasites are highly resistant to common disinfectants and can persist in water for weeks (Ryan *et al.*, 2014). They are shed in large numbers in feces by infected humans and animals, leading to water contamination through waste disposal and erosion, which can transport the oocysts to nearby streams or rivers.

Cryptosporidium is a significant contributor to diarrhea in children, whether or not they are infected with the human immunodeficiency virus (HIV), particularly in developing nations (Javier *et al.*, 2008). Diarrhea can often result in stunted growth in children, as it is linked to poor nutrient absorption and decreased appetite (UNICEF, 2019). This infection tends to be more prevalent in children under five years old compared to those older than five years (Sha *et al.*, 2003; Huang *et al.*, 2009). Diarrhea is a leading cause of over 3.1 million deaths annually among children under 15 years old, predominantly in developing regions. Environmental contamination with infectious oocysts heightens the risk of transmission to susceptible individuals (Huang *et al.,* 2009).

This study aims to investigate the prevalence of cryptosporidium infection among children attending Maryam Abacha Women and Children Hospital (MAWCH), Sokoto. By detecting the parasite and the factors contributing to its spread, this study seeks to provide a comprehensive understanding of the current situation. The findings are expected to enhance effective diagnosis treatment and improve the overall health and well-being of children.

MATERIALS AND METHODS

Study Area

The research was carried out at the Maryam Abacha Women and Children Hospital in Sokoto, situated in Sokoto North, Sokoto State. This region has an estimated population of about 4 million people (NPC, 2007). Sokoto State is positioned in the far North-Western part of Nigeria, spanning from longitude 4° to 6° east and latitude 10° to 14° north of the equator. It consists of 23 local government areas and shares borders with Niger Republic to the North, Kebbi State to the South-West, and Zamfara to the East. The total land area of Sokoto State covers approximately 32,000 square kilometers, with a population of around 3,696,999 inhabitants. The climate in Sokoto is semi-arid, characterized by Sudan Savannah vegetation, with annual rainfall ranging between 500-1300mm and temperatures varying from 15°C to over 40°C on warm days (Oche *et al.,* 2019).

Sample Size Determination

The sample size for this research was calculated based on the prevalence of 3.8% obtained from a previous study (Banwat *et al.,* 2003).

The number of sample sizes was determined using the formula;

$$n = \frac{Z^2PQ}{d^2}$$

n = ?
Z = 1.96
P = 3.8% (0.038) (Banwat *et al.,* 2003).
Q = (1-0.038) = 0.962
d = 5% (0.05)

$$n = \frac{(1.96)^2 \times 0.962 \times 0.038}{(0.05)^2}$$

n= 56.2~57

Therefore, 57 diarrheic and 57 non-diarrheic samples were collected for the study.

57+57 =114

Therefore, the total number of samples collected was 114 samples.

Research Ethical Approval

Approval was granted by the Ethics and Research Committee of Maryam Abacha Women and Children Hospital in Sokoto in adherence to the guidelines outlined in the code of ethics for Biomedical Research involving Human subjects (Ref. No.: MAWCH/1580/V.IV).

Informed Consent

The significance and advantages of the study were communicated to all parents or guardians of the participants to ensure their voluntary involvement, and written informed consent was obtained from each participant's relative.

Questionnaire Administration

A well-structured questionnaire was administered to parents/guardians of the children to assess socio-demographic characteristics as well as the possible risk factors associated with cryptosporidiosis in children.

Collection of Samples

stool samples were collected using a clean, dry, wide-mouth container, and participants were instructed not to mix urine with the stool sample. The lids were securely fastened, and the samples were gently blended by rocking the container. The samples were stored at room temperature, properly labeled with the participant's details, including identification number and time of collection, and processed within 24 hours. For infants, the diaper was lined with plastic before use. The stool was then scraped from the plastic lining and placed in the appropriate container(s). Parents or guardians were informed that directly scraping stool from the diaper is not an ideal sampling method (Cheesbrough, 2006).

Stool Sample Examination Using Formol Ether Concentration Technique

A glass slide was designated for each participant. Using a Pasteur pipette, 1ml of thoroughly mixed watery stool sample was dispensed into a screw-capped test tube.

Approximately 3-4ml of 10% v/v formol water was added to the tube, which was then capped and shaken well to ensure thorough mixing. The emulsified feces were filtered, and the resulting suspension was collected in a beaker. This suspension was subsequently transferred into a polypropylene conical centrifuge tube. 3ml of diethyl ether was introduced into the tube, sealed, and mixed for 1 minute. Due to the flammability of ether and ethyl acetate, caution was exercised by maintaining a safe distance from any open flames during this process. Cotton wool was wrapped around the tube's opening, the stopper was loosened, and the mixture was immediately centrifuged at 1000rpm for 1 minute. Following centrifugation, any fecal debris adhering to the tube's walls was dislodged using a stick. The tube was inverted to discard the ether, fecal debris, and formol water, allowing the sediment to remain. Once the tube was upright again, any residual fluid on the tube's walls was allowed to drain to the bottom before tapping the tube to resuspend the sediment. The sediment was then transferred onto two grease-free glass slides, with one designated for a wet preparation and the other for a smear that was applied thinly and evenly, avoiding excessive thickness or gaps. The slides were left to dry before undergoing staining. Prior to staining, the smear was fixed in methanol for two minutes, after which the slides were placed on a staining rack for appropriate staining procedures (Ochei and Kolhatkar, 2007).

Stool Sample Examination Using Modified Ziehl-Neelsen Staining Technique

The modified Ziehl-Neelsen (Cold Acid-Fast) staining method was employed to detect *Cryptosporidium* species. Following fixation in methanol for 2 minutes, the smear was allowed to air-dry thoroughly on the staining rack. Subsequently, the dried and fixed smear was coated with carbol fuchsin stain for 15 minutes at room temperature without the application of heat. The excess stain was washed away, followed by decolorization using 1% acid alcohol for 10-15 seconds, and then rinsing with water. Counterstaining with methylene blue was performed for 30 seconds, after which the slide was rinsed under running water and placed on a draining rack (Cheesbrough, 2006). Once the slides were completely dry, a drop of immersion oil was applied to each slide and examined using a compound microscope at ×100 magnification for *Cryptosporidium* oocysts.

Statistical Analysis

Data obtained was analyzed using a statistical package for social sciences (SPSS) version 23 (IBM, Chicago, USA). All tests were considered statistically significant at $P \leq 0.05$. Chi-Square test was used to determine the association between *Cryptosporidium* infection with age, gender and other demographic characteristics of the study participants.

RESULTS

A total of 114 children took part in the study. Out of these, 56 (49.1%) were females, and 58 (50.9%) were males. Additionally, 57 (50%) of the children had diarrhea, while 57 (50%) did not. When categorized by age groups, the distribution was as follows: 62 children (54.4%) were aged 0-1 years, 28 (24.6%) were aged 2-3 years, 9 (7.9%) were aged 4-5 years, 5 (4.4%) were aged 6-7 years, and 10 (8.8%) were aged 8-9 years (Table 1).

Table 2 displays the prevalence of Cryptosporidiosis among children in the study area concerning gender and age distribution. Out of the 114 samples analyzed, 81 (71.1%) tested positive for Cryptosporidiosis. When looking at gender-specific infection rates, males had a higher rate of infection at 45 (39.5%) compared to females, who showed a total of 36 (31.6%) infections. In terms of age groups, the highest infection rate was observed in the 0-1-year-old group (36.0%), followed by the 2-3-year-olds (18.4%), 8–9-year-olds (7.9%), and both 4-5- and 6-7-year-olds (4.4%) respectively.

Children who consumed properly cooked vegetables had a higher prevalence of 34.2%, followed by those who ate undercooked vegetables at 20.2%, raw vegetables at 2.6%, and those who did not eat vegetables at 14.0%. Regarding water sources, children who accessed water from Taps and boreholes had the highest prevalence at 39.5%, while those using water from the river and well showed rates of 21.9% and 9.6%, respectively. A statistically significant association ($p = 0.001$) was observed between sources of water and Cryptosporidium infection. In terms of contact with animals, the prevalence was higher among children with animal contact at 36.8% compared to those without at 34.2%. The type of toilet facility also influenced prevalence, with pit users at 36.8%, flush users at 29.8%, and bush users at 2.6%. Furthermore, the prevalence of Cryptosporidiosis was significantly higher ($p = 0.002$) among diarrheic participants at 42.1% compared to non-diarrheic participants at 28.9% (Table 3)

Table 1: Demographic Characteristics of the Study Participants

Age group (years)	Gender		Total (%)
	Male (%)	Female (%)	
0-1	35 (30.7)	27 (23.7)	62 (54.4)
2-3	13 (11.4)	15 (13.2)	28 (24.6)
4-5	2 (1.8)	7 (6.1)	9 (7.9)
6-7	2 (1.8)	3 (2.6)	5 (4.4)
8-9	6 (5.3)	4(3.5)	10 (8.8)
Total	58 (50.9)	56 (49.1)	114 (100)

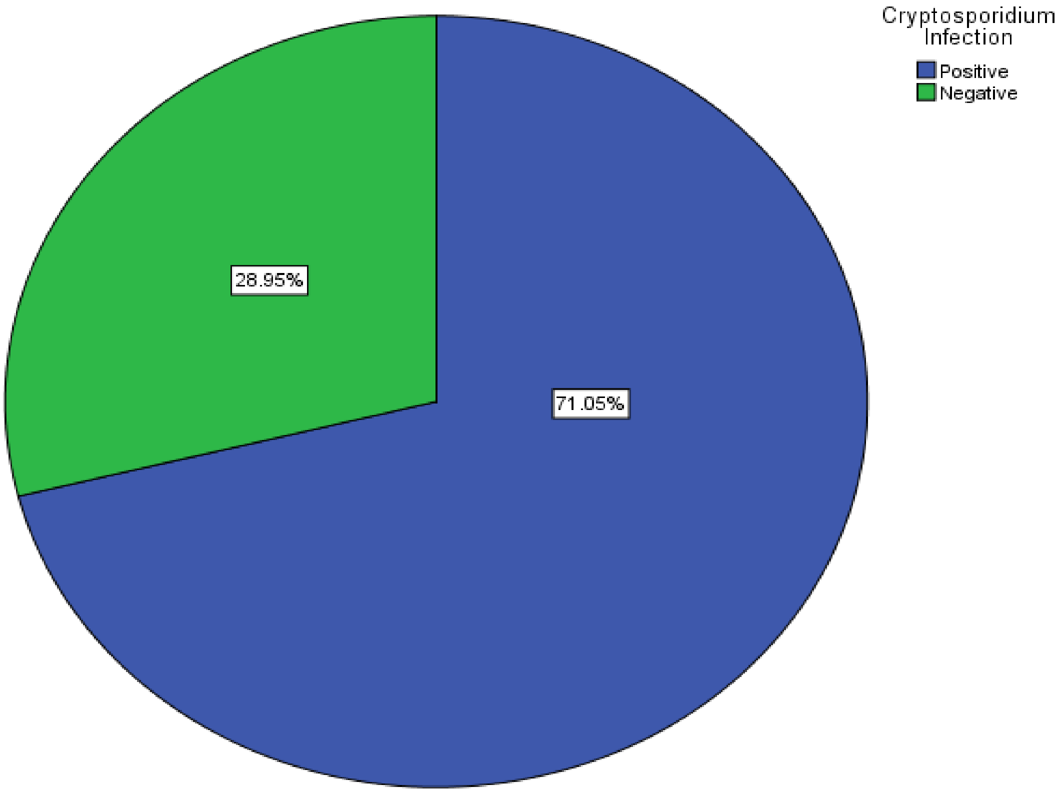

Figure 1: Prevalence of Cryptosporidium Infection among Children in the Study Area

Table 2: Prevalence of *Cryptosporidium* Infection in Relation to Gender and Age

Variables	*Cryptosporidium* Infection		Total (%)	P value
	Pos (%)	Neg (%)		
Gender				0.117
Male	45 (39.5)	13 (11.4)	58 (50.9)	
Female	36 (31.6)	20 (17.5)	56 (49.1)	
Total	81 (71.1)	33 (28.9)	114(100)	
Age group (years)				0.216
0-1	41 (36.0)	21 (18.4)	62 (54.4)	
2-3	21 (18.4)	7 (6.1)	28 (24.6)	
4-5	5 (4.4)	4 (3.5)	9 (7.9)	
6-7	5 (4.4)	0 (0.0)	5 (4.4)	
8-9	9 (7.9)	1 (0.9)	10 (8.8)	
Total	81 (71.1)	33 (28.9)	114(100)	

Table 3: Association of Cryptosporidiosis with Potential Risk Factors

Variables	Number Examined	No. Positive	Percentage (%)	P value
Vegetable Consumption				0.134
Properly cooked	62	39	34.2	
Undercooked	28	23	20.2	
Raw	5	3	2.6	
Do not eat vegetables	19	16	14.0	
Water source				0.001*
Tap and borehole	75	45	39.5	
River	27	25	21.9	
Well	12	11	9.6	
Contact with animals				0.536
Yes	57	42	36.8	
No	57	39	34.2	
Toilet facilities				0.490
Pit toilet	64	44	38.6	
Water closet	47	34	29.8	
Bush	3	3	2.6	
Diarrhea				0.002*
Yes	57	48	42.1	
No	57	33	28.9	

* Statistically significant P value < 0.05.

DISCUSSION

The overall prevalence of Cryptosporidium infection in this study is 71.1%, which is quite worrisome and a reflection of the high rate of intestinal parasites among children in endemic regions. This result is similar to the 62.5% rate reported by Shinkafi *et al.* (2017) among primary school children in Wamakko Local Government Area of Sokoto state, Nigeria. However, this finding is significantly higher than rates reported in previous studies, such as 4.6% in Zaria (Gambo *et al.*, 2014), 10.7% in Maiduguri (Askira *et al.*, 2020), 16.6% in Sokoto (Saulawa *et al.*, 2016), and 27% in Jos (Pam *et al.*, 2013). Our study also contrasts with findings from other African countries. For instance, the prevalence rates reported in Sudan, Benin, and Cameroon were 27.1% (Tamomh *et al.*, 2020), 5.8% (Ogouyèmi-Hounto *et al.*, 2017), and 13.4% (Tombang *et al.*, 2019) respectively.

Additionally, the prevalence rate was reported at 3.1% in Israel (Shaposhnik *et al.*, 2019). The disparity in prevalence rates may stem from limited public awareness of the infection among the study participants, particularly since many participants are infants. Some practices by the parents, such as traditional force-feeding through oral drenching with bare hands to ensure adequate food intake for proper growth, may contribute to the exposure of participants to Cryptosporidiosis. The risk of transmission of contaminative infections like Cryptosporidiosis from infected adults to infants and children is heightened when bare hands (often not properly washed) are used for feeding babies. Furthermore, in the study area, it is common for parents to begin weaning their infants at around three to four months of age, with some starting as early as the first two months of life. This early weaning practice typically involves giving water and traditional foods that are high in carbohydrates but low in protein. These dietary choices may not provide sufficient support for the development of a robust immune system without the addition of supplements, as noted by Ayinmode *et al.* (2018).

Gender-specific infection rates revealed that males had a higher infection rate compared to their female counterparts. However, the difference was not statistically significant (p = 0.117). The higher prevalence of infections among male participants could be attributed to factors such as differences in caregiving practices favoring females and the tendency for females to spend more time indoors. This finding aligns with the results of studies by Askira *et al.* (2020) and Tamomh *et al.* (2020) but differs from the study by Gambo *et al.* (2014), where the prevalence of Cryptosporidiosis was slightly higher in females than males.

When analyzing the prevalence of *Cryptosporidium* infection by age group, it was found that children aged 0-1 years had the highest infection rate. This could be due to the fact that the majority of their participants are over 1 year old. While infections can impact individuals across all age groups, certain cultural beliefs in Nigeria contribute to the underdiagnosis of Cryptosporidiosis in children aged 0-1 years.

In some rural communities, there is a belief that diarrhea is a natural occurrence linked to developmental milestones like teething and crawling. Some also believe that a heavy infant develops diarrhea to lose weight and facilitate walking, while others associate diarrhea with the appearance of the anterior fontanel. These cultural perceptions often deter individuals from seeking medical help, leading to undiagnosed cases of Cryptosporidiosis among infants aged 0-1 years. This observation corresponds with findings from studies conducted by Gambo *et al.* (2014) and Ogouyèmi-Hounto *et al.* (2017) but differs from the results reported by Shinkafi *et al.* (2017) and Askira *et al.* (2020), which indicated higher prevalence rates among 6-8-year-olds and 2.5-3.5-year-olds, respectively.

A higher prevalence was found among individuals who consume properly cooked vegetables, while those who consume raw vegetables had the lowest prevalence. This difference was not statistically significant (p = 0.134) and contrasts with the findings reported by Saulawa *et al.* (2016). This variation may be attributed to the multiple transmission routes of Cryptosporidiosis, which include airborne transmission, the fecal-oral route, and contact with animals.

A statistically significant difference (p = 0.001) was observed concerning the water source. The highest prevalence was documented in children who obtained water from taps and boreholes, while the lowest prevalence was found in children who used well water. Despite undergoing water treatment processes, *Cryptosporidium* species can withstand common treatments like chlorine disinfection. Poor hygiene practices, such as handling contaminated materials with unwashed hands and using unclean containers to store water for child consumption, can contribute to the transmission of waterborne infections like Cryptosporidiosis from infected adults to children. This finding aligns closely with the study conducted by Tamomh *et al.* (2020).

In terms of contact with animals, the highest prevalence was observed in children who had such contact. This finding is consistent with a study by Gambo *et al.* (2014) but contrasts with the findings of Anejo-Okopi *et al.* (2016). This variability supports the notion that the epidemiological distribution of Cryptosporidiosis remains unclear due to various modes of transmission, including human-to-human, animal-to-human, foodborne, waterborne, and airborne routes.

Regarding toilet facilities, the highest prevalence was found in children using pit toilets, and the least was found in those using bushes. This differs from the report by Gambo *et al.* (2014). This discrepancy may arise from inadequate hand washing practices after defecation or before meals, as well as other unhygienic behaviors like finger sucking, fingernail biting, and picking up food from the ground or floors

In relation to diarrhea, a statistically significant difference (p = 0.002) was observed. The highest prevalence was found among participants with diarrhea compared to those without diarrhea. Diarrhea often manifests as a complication of cryptosporidiosis, as previous research has highlighted *Cryptosporidium* infection as a common cause of persistent diarrhea in developing countries, responsible for approximately one-third of cases. This finding is in line with studies conducted by Shaposhnik *et al.* (2019), Saulawa *et al.* (2016), and Tamomh *et al.* (2020).

CONCLUSION

The study revealed a high prevalence of *Cryptosporidium* infection in the study area. Findings indicate that the highest prevalence of cryptosporidiosis was observed in younger children aged 0-1 years compared to older children, as well as in males compared to females. Similarly, a higher infection rate was noted in children with diarrhea compared to those without diarrhea. The presence of diarrhea and the sources of drinking water were significantly linked to cryptosporidiosis in children. This underscores the fact that *Cryptosporidium* is a major contributor to diarrhea in children.

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