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# Prevalence of *Cryptosporidium* Coproantigens in Humans in Sokoto State, North Western Nigeria

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**ABSTRACT**

*Human cryptosporidiosis caused by Cryptosporidium parasite has been recognised worldwide as the most common cause of protozoal diarrhea leading to significant morbidity and mortality in industrialized nations and developing countries. However few epidemiological studies of the parasite has been conducted in this study area using immunoassay diagnostic tools. This study was a prospective cross sectional, hospital-based study carried out to detect the prevalence of Cryptosporidium species copro-antigens in humans in Sokoto state, Northwestern Nigeria using a commercially manufactured Cryptosporidium Copro-Enzyme Linked Immunosorbent Assay (Copro-ELISA) kit (Savyon™ Diagnostics Inc., Israel), which is 100% sensitive and specific for Cryptosporidium. Three hundred and sixty eight (368) human faecal samples were collected and analyzed, 61 (16.6%) were positive for the parasite. Children ≤5 years of age were found to have significantly ($\chi2$= 7.587; p= 0.005) higher prevalence (28.1%) than older patients (12.5%). Symptoms including diarrhea ($\chi2$= 8.590; p= 0.003), abdominal pain ($\chi2$= 20.241; p= <0.0001) and fever ($\chi2$= 5.444; p= 0.019) were demonstrated to be significantly associated with cryptosporidiosis. Cryptosporidium infection in Sokoto state had significant association (p< 0.05) with consumption of raw vegetables ($\chi2$= 6.685; p= 0.0097), while those that consume the raw vegetables had 22.2% (45/203) prevalence against 9.7% (16/165) that did not. However, the results shows no significant difference between the prevalence of Cryptosporidium copro-antigens and the following variables in the study population; sex ($\chi2$= 0.122; p= 0.730); educational background ($\chi2$= 6.233; p= 0.101); stool consistency ($\chi2$= 1.107; p= 0.293); water source ($\chi2$= 4.326; p= 0.228); animal contact ($\chi2$= 1.503; p= 0.220) and toilet system ($\chi2$= 5.049; p= 0.080). These findings highlighted the presence of Cryptosporidium infection among humans in Sokoto state, Northwestern Nigeria.*

**Keywords**: Copro-antigens, *Cryptosporidium*, ELISA, Humans, Nigeria, Prevalence, Sokoto state

## INTRODUCTION

Human cryptosporidiosis caused by *Cryptosporidium* parasite has been recognized worldwide as the most common cause of protozoal diarrhea leading to significant morbidity and mortality in industrialized nations and developing countries [1]. Although person-to-person transmission has been considered the major route of *Cryptosporidium* transmission, zoonotic transmission of this protozoan may also occur [2]. *Cryptosporidium* oocysts may remain viable in water for over 140 days [3]. The oocysts are very resistant to the most common disinfectants [4] making them difficult to be destroyed by conventional chlorination treatment. *Cryptosporidium* is a morphologically identical but genetically different multiple genotypes parasite [5, 6]. In humans, *Cryptosporidium hominis* (*C. hominis*) and *Cryptosporidium parvum* (*C. parvum*) are the most common causes for the majority of infections [7]. *C. hominis* genotype is exclusively found in humans, whereas *C. parvum* is found in humans, domestic, and wild animals [5, 6]. Cryptosporidiosis is a major cause of diarrhea and generally causes self-limited watery diarrhea in

immuno-competant patients or chronic severe diarrhea in immunocompromised individuals [8, 9]. Previous studies in various tropical countries have shown that children of 5 years of age and below are the most susceptible to *Cryptosporidium* infection, with the reported incidence ranging from 1.1 to 18.9% [10, 11, 12].

*Cryptosporidium* is an obligate intracellular parasite that infects the epithelial lining of luminal surfaces of gastrointestinal and respiratory tracts in a wide array of hosts. The parasite is ingested as oocysts which undergoes excystation to sporozoites that parasitise the host. Infection can occur in oesophagus and any portion of gastrointestinal tract can be involved; it usually starts in the lower small intestine. Other areas include the gall bladder, bile ducts, pancreas and respiratory tract. The infection provokes symptoms such as abdominal cramps, diarrhea, vomiting, loss of appetite, low grade fever, generalized malaise and nausea [13]. While the infection can resolve without intervention in immuno-competant individuals, cryptosporidiosis is increasingly becoming a major public health threat as an opportunistic infection in immunosuppressed and immunocompromised individuals, especially in HIV/AIDS [14, 15]. In immuno-competant individuals, the parasite is localized in the distal small intestine and proximal colon, but occurs throughout the gut, biliary and respiratory tracts in immunocompromised hosts. Defects in innate, humoral or cellular immunity in a patient infected with *Cryptosporidium* results in severe or prolonged illness. The life threatening potential of *Cryptosporidium parvum* in immunocompromised and immunosuppressed individuals has increased the importance of cryptosporidiosis as a global public health problem [16].

Diagnosis of cryptosporidiosis is usually carried out by conventional method of examination of fecal smears or concentrates using acid-fast stains. These methods are tedious, time consuming and require experienced personnel to identify the organism. The need for a test that is rapid, easy to perform and interpret, and cost effective has led to the development of immunoassay techniques like immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) for the diagnosis of cryptosporidiosis [17]. Commercial *Cryptosporidium* ELISA kits which are rapid, convenient and easy to use are now available as diagnostic tools for this disease. However, neither microscopy nor ELISA can differentiate *Cryptosporidium* species [18] and genotypes/subtypes; *Cryptosporidium s*pecies can only be identified by polymerase chain reaction (PCR)-based techniques [19]. In spite of the limitations of the ELISA technique in the investigation of *Cryptosporidium parvum*, it is still relied on for epidemiological surveys in humans due to higher sensitivity and ease of use compared to microscopy [20], especially in developing nations where molecular diagnostic tools are not readily available. In Nigeria, the few available reports of cryptosporidiosis in animals were based on microscopy of stained oocysts in faeces [21, 22], with none utilizing the immunoassay method of investigation. The present study was conducted to find the prevalence of *Cryptosporidium* Copro-antigens using a commercially available *Cryptosporidium* Copro-ELISA kit and the associated risk factors in humans in Sokoto state, Northwestern Nigeria.

## MATERIALS AND METHODS

### Study Design

This study was a prospective cross-sectional, hospital-based study carried out between December, 2013 and October, 2014.

### Study Area

Sokoto state is geographically located at the North Western part of Nigeria between longitude $11^0$ 30' to $13^0$ 50' East and latitude $4^0$ to $6^0$ 40' North. The state shares common borders with Niger Republic to the North, Kebbi State to the South and Zamfara State to the East [23]. The state falls in the dry Sahel surrounded by sandy Sudan type Savannah [24].

Four Local Government Areas (LGA) were selected using simple random sampling method from the four agricultural zones of Sokoto state (one LGA from each zone) as outlined by the State Ministry of Agriculture (Figure 2.1). They included Yabo, Sokoto, Wurno and Gwadabawa LGA's.

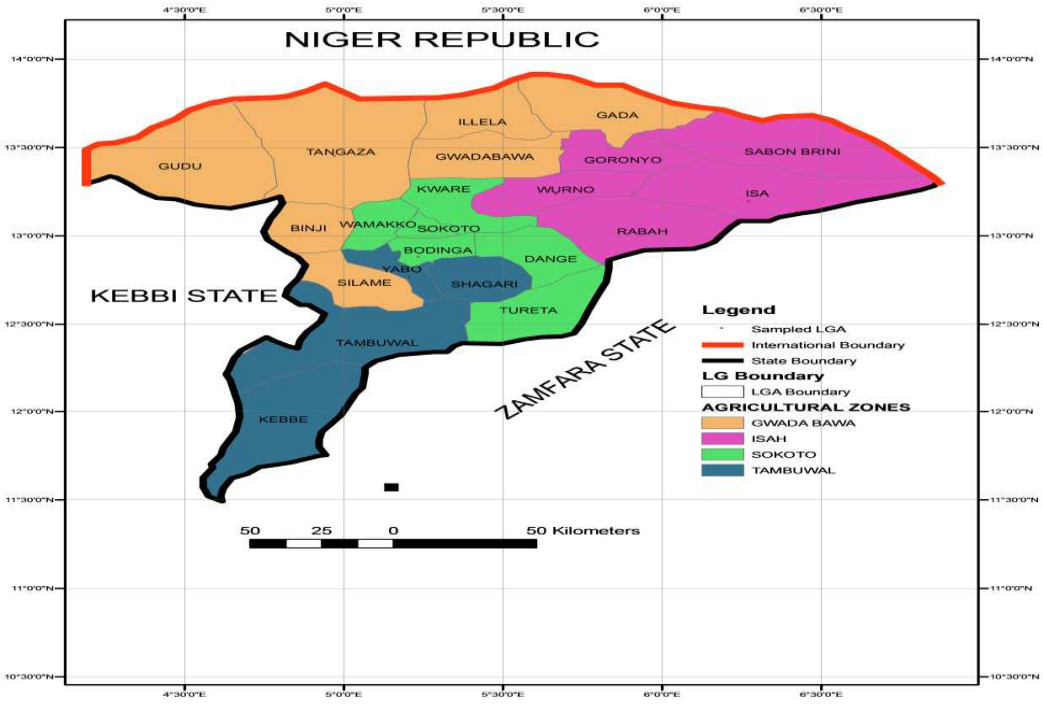


**Figure 2.1: Sokoto state map showing the four Agricultural zones of the state**
*Source: Sokoto state Ministry of Agriculture and Natural Resources.*

**Target Population**
This study was carried out among patients presented with diarrhea, immunocompromised conditions i.e HIV, Hepatitis, children ≤ 5 years of age, all consented to take part in the study. Prior to stool sample collection, ethical clearance was sought for and issued by the Ethical Committee of Sokoto State Ministry of Health. Samples were then collected from patients found at the four General Hospitals of each of the selected Local Government Areas of the four Agricultural zones of Sokoto state, namely: Wurno, Yabo, Gwadabawa General Hospitals and Sokoto Specialist Hospital, Sokoto state.

**Inclusion Criteria**
All patients that were presented with diarrhea, immunocompromised individuals or children ≤ 5 years of age in the hospital and who gave their consent to participate in the study were sampled.

**Structured Questionnaire**
Structured questionnaire was used to collect demographic data and patient's information on age, sex, feeding, water source, educational background, sanitation and symptoms.

**Stool Sample Collection**
Three hundred and Sixty Eight (368) freshly voided faeces were collected in a wide-mouthed sample containers. Ten percent (10%) formalin (twice the volume of the faeces) was added to each container for preservation (as directed by the Copro-ELISA kit manufacturer) before being transported to the Central Research Laboratory, Usmanu Danfodiyo University Sokoto, Nigeria, where the samples were analyzed.

**Detection of *Cryptosporidium* Copro-antigens by Copro-ELISA**
The detection of *Cryptosporidium* species coproantigens in the samples was done using a commercially available Copro-ELISA kit for faecal sample (*Cryptosporidium* Copro-Enzyme Linked Immunosorbent Assay™ for Humans manufactured by Savyon® Diagnostics Ltd., Ashdod, Israel). The procedure was carried out according to the manufacturer's instruction.

0.1g of each faecal sample was homogenized in 300µl of sample dilution buffer and centrifuged. 200µl of Negative and 100 µl of positive controls, and 100 µl of each of the sampled specimens were added in the wells of microtitre plate coated with anti-*Cryptosporidium* species antibodies and incubated at room temperature for 1 hour. The plate

was washed five times with a washing buffer (300 µl), 100 µl of HRP-Conjugate was added and incubated at room temperature for one hour and washed five times. 100 µl of TMB-Substrate and incubated for 15 minutes, a 100 µl of stop solution was added to each of the well and read using the ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA) at 450/620nm.

Samples with Optical Density (OD) reading higher than 1.0 is positive, while those with OD lesser than 1.0 were reported as negative for *Cryptosporidium* coproantigens.

**Statistical Analysis**

The data was computed and analyzed using IBM Statistical Package for Social Sciences (SPSS) on Windows 8 PC. Data were summarized using frequency tables. Univariate association between *Cryptosporidium* species infection and possible risk factors were assessed using Pearson's Chi-square ($\chi 2$) test. The odds ratio (OR) and the corresponding 95% confidence interval (95% Cl) were calculated to measure the strength of association between variables and occurrence of *Cryptosporidium* oocysts. P-values $\leq 0.05$ were considered significant.

## RESULTS

**Demographic Factors of the Study Population**

Table 1 shows the demographic factors in this study. The prevalence of *Cryptosporidium* copro-antigens in this study population was 16.6% (61/368). Sex was not significantly associated with the occurrence of cryptosporidiosis in the population ($\chi 2$=0.122; p= 0.730). Male has the highest prevalence (17.5%) as against females (15.2%) with an Odds ratio of 0.860 and 95% Confidence Interval (95% CI) of 0.498-1.520. However, assessment of the relationship between age and *Cryptosporidium* infection shows that there was a significant association ($\chi 2$= 7.587; p= 0.005), though a prevalence of 28.1% (27/96) was observed in patients of ≤5 years as against a prevalence of 12.5% (34/272) in patients > 5 years. The Odds ratio was observed to be 0.444 and 95% CI is 0.255-0.775 (Table 1)**.**

**Table 1: Demographic Effects on the Prevalence of *Cryptosporidium* Copro-antigens in Humans in Sokoto state, Nigeria**

| Factors | No. tested | No. Positive (%) | $\chi 2$ | p-value | Odds ratio | 95% C.I |
|---|---|---|---|---|---|---|
| **Sex** | | | | | | |
| Male | 217 | 38 (17.5) | 0.122 | 0.730 | 0.860 | 0.498-1.520 |
| Female | 151 | 23 (15.2) | | | | |
| **Age (Years)** | | | | | | |
| ≤5 | 96 | 27 (28.1) | 7.587 | 0.005* | 0.444 | 0.255-0.775 |
| >5 | 272 | 34 (12.5) | | | | |
| **Education** | | | | | | |
| Primary | 163 | 21 (12.9) | 6.223 | 0.101 | | |
| Secondary | 47 | 14 (29.8) | | | | |
| Tertiary | 28 | 7 (25.0) | | | | |
| None | 130 | 19 (14.6) | | | | |

**= p<0.05; χ2= Chi-square; C. I = Confidence Interval; P= Level of Significance*

**Association between Cryptosporidiosis and Symptoms Reported**

Table 2 shows the relationship, if any, between symptoms reported by the patients and the prevalence of *Cryptosporidium* copro-antigens in stool samples. A prevalence of 21.4% (53/248) was found in patients with diarrhea and 6.7% (8/120) was seen in patients without diarrhea. There was a significant association ($\chi 2$= 8.590; p= 0.003) between diarrhea and human cryptosporidiosis. The Odds ratio (OR) was 0.312 and 95% CI is 0.144 - 0.677. Stool consistency was not significantly associated ($\chi 2$= 1.107; p= 0.293) with cryptosporidiosis, while abdominal pain was significantly associated ($\chi 2$= 20.241; p<0.0001) with the occurrence of *Cryptosporidium* copro-antigens in humans with an Odds ratio of 0.268; 95% CI: 0.149-0.480. Out of the 279 patients that had fever, 55 (19.7%) had cryptosporidiosis indicating a significant association ($\chi 2$= 5.444; p= 0.019) between fever and the presence of *Cryptosporidium* copro-antigens (Table 2).

**Table 2: Symptoms Associated with the Prevalence of *Cryptosporidium* Copro-antigens in Humans in Sokoto state, Nigeria**

| Factors | No. tested | No. Positive (%) | χ2 | p-value | Odds ratio | 95% C.I |
|---|---|---|---|---|---|---|
| **Diarrhea** | | | | | | |
| Present | 248 | 53 (21.4) | 8.590 | 0.003** | 0.312 | 0.144-0.677 |
| Absent | 120 | 8 (6.7) | | | | |
| **Stool Consistency** | | | | | | |
| Watery | 193 | 37 (19.2) | 1.107 | 0.293 | 0.715 | 0.412-1.244 |
| Loose | 175 | 24 (13.7) | | | | |
| **Abdominal Pain** | | | | | | |
| Present | 137 | 42 (30.7) | 20.241 | <0.0001** | 0.268 | 0.149-0.480 |
| Absent | 231 | 19 (8.2) | | | | |
| **Fever** | | | | | | |
| Present | 279 | 55 (19.7) | 5.444 | 0.019* | 0.342 | 0.142-0.821 |
| Absent | 89 | 6 (6.7) | | | | |

**= p<0.05; **= p≤ 0.01: χ2= Chi-square; C. I = Confidence Interval; P= Level of Significance*

### Association between the Detection of *Cryptosporidium* Copro-antigens and Risk Factors

Table 3 shows the possible risk factors studied. Water source (χ2= 4.326; p= 0.228), animal contact (χ2= 1.503; p= 0.220) and toilet system (χ2= 5.049; p= 0.080) were not significantly associated with the *Cryptosporidium* infection in humans. Consumption of raw vegetable was significantly associated (χ2= 6.685; p= 0.0097) with the occurrence of cryptosporidiosis in humans with an Odds ratio of 0.437; 95% CI: 0.238-0.802 (Table 3).

**Table 3: Risk Factors Associated with the Prevalence of *Cryptosporidium* Copro-antigens in Humans in Sokoto State, Nigeria**

| Factors | No. tested | No. Positive (%) | χ2 | p-value | Odds ratio | 95% C.I |
|---|---|---|---|---|---|---|
| **Animal Contact** | | | | | | |
| Yes | 267 | 39 (14.6) | 1.503 | 0.220 | 1.491 | 0.843-2.639 |
| No | 101 | 22 (21.8) | | | | |
| **Consumption of Raw Vegetables** | | | | | | |
| Yes | 203 | 45 (22.2) | 6.685 | 0.0097** | 0.437 | 0.238-0.802 |
| No | 165 | 16 (9.7) | | | | |
| **Water Source** | | | | | | |
| Pipe-borne | 76 | 12 (15.8) | 4.326 | 0.228 | | |
| Well | 204 | 27 (13.2) | | | | |
| Bore-hole | 18 | 4 (22.2) | | | | |
| Stream | 70 | 18 (25.7) | | | | |
| **Toilet System** | | | | | | |
| Pit latrine | 312 | 47 (15.1) | 5.049 | 0.080 | | |
| Water cistern | 40 | 13 (32.5) | | | | |
| In the field | 9 | 1 (11.1) | | | | |

**= p<0.05; **= p≤ 0.01: χ2= Chi-square; C. I = Confidence Interval; P= Level of Significance*

## DISCUSSION

The prevalence of 16.6% (61/368) obtained in this study by using a commercially prepared *Cryptosporidium* Copro-ELISA kit was similar to the findings in Iraq [25] and in Egypt [26]. The result contrasted with some studies conducted at Kaduna, Nigeria [27, 28], Jos, Nigeria [29] and Ogun [30]. This observation confirms the existence of cryptosporidiosis in humans in the study area.

The study also showed a higher prevalence in male than in females which contradicts the reports of Gambo [28] and Okojokwu [27]. The reason for the observed difference was not clear. Prevalence of cryptosporidiosis was higher among patients in age group ≤ 5 years (28.1%) than it was in the > 5 years (12.5%). This findings revealed that there was statistically significant (χ2 = 7.587, p = 0.005) association between age and cryptosporidiosis with children in the age group ≤ 5 years being more prone to contact the infection than their older counterparts. The odds ratio (OR) of 0.444 (95% CI = 0.255 – 0.775) indicates that children in the age group ≤ 5 years were twice more predisposed or liable to contract cryptosporidiosis than those who were older. This may be due to the fact that children in the ≤ 5 years group have been found to be more vulnerable when exposed to contaminated environment, food and water. Immunity is less than optimal at both ends of life, that is, ≤ 5 years and in the elderly. Newborns appear to have less T-cell functions and antibodies are acquired by the transfer of IgG from their mothers through the placenta. This maternal IgG decay overtime with little remaining by 3 – 6 months of age; and the risk of infection in children is

higher after the age of 6 months [31]. These findings are in tandem with the works of Nwabuisi [32], Nyamwange [15] and Okojukwu [27] which showed that *Cryptosporidium* infection had higher prevalence in children than in adults. Apart from the fact that children have higher susceptibility to infections attributable to immature immune system, the possibility of faecal-oral transmission of the infections is higher in children aged ≤ 5 years since they are more likely to practice coprophagy and more unlikely to practice good hygienic habits [32]. There was no statistical association ($\chi2$= 6.223; p= 0.101) existed between *Cryptosporidium* infection and educational status of the patients or their care-givers (for patients who have not attained school age).

Diarrhea was found to be significantly ($\chi2$= 8.590; p= 0.003) associated with the presence of cryptosporidiosis among the study population. The prevalence was higher in patients with diarrhea (21.4%) than those without diarrhea (6.7%) in this study. This report is in contrast with the report of Okojokwu [27] in Kaduna state, Nigeria and El-Helaly [26] at Egypt. Previous researchers noted that *Cryptosporidium* infection is among the common causes of persistent diarrhea in developing countries causing approximately one third (1/3) of cases [33, 34]. Stool consistency showed no association ($\chi2$= 1.107; p= 0.293) with *Cryptosporidium* infection. Watery stool (19.2%) was found to be of higher prevalence than loose stool (13.7%). This may be due to the fact that infection is characterized by defects in intestinal permeability [27]. Increased permeability may result in decreased absorption of fluids and electrolytes as well as solute fluxes into the gut. Similar defects have been noted by Zhang [35] in children with cryptosporidiosis. The prevalence of cryptosporidiosis among patients with watery stool (19.2%) in this study was low compared to the prevalence of 39.66% reported by Zhang [35] and 40.7% by El-Helaly [26]. The wide variation in the reported and observed prevalence of cryptosporidiosis may be attributed to geographical locations and the type of method used for the detection of oocysts [27]. Adesiji [36] noted that prevalence of cryptosporidiosis tends to vary from one locality to another and from one country to another, though this variation is predicted on the extent of contamination of water, food and animal contact; all these are considered important factors for dissemination of the coccidian parasite. Sample size could also play a prominent role in variation of the prevalence since the study of Sanein [37] with 200 samples (18 cases) had prevalence of 9.0%, 6.0% (36/600) [38]; 5.0% (30/600) [27], while our findings in this study showed 16.6% (61/368) prevalence. The prevalence observed in this study is in contrast with the higher prevalence reported from Zaria [39], Jos [40] and Imo state, Nigeria [41]. The prevalence reported in Imo state could be attributed to the inclusion of immunocompromised individuals in the samples whom are known to be highly vulnerable to opportunistic infections such as cryptosporidiosis [28]. The association between gender and cryptosporidiosis was significant ($\chi2$= 7.587; p= 0.05) which was higher in males (17.5%) than in females (15.2%). This observation was consistent with the earlier made in this regard by researchers [32, 42, 37], but was contradicted in the reports of Okojokwu [27] who reported 6.4% higher. This observed disparity in prevalence could be attributed to the population under study.

Abdominal pain was significantly ($\chi2$= 20.241; p< 0.0001) associated with the presence of *Cryptosporidium* copro-antigens. The manifestation of abdominal pain could be attributed to the production of chemokines and cytokines by the cells of the infected intestines. The infection could also up-regulate the expression of cyclooxygenase-2, production of prostaglandins by the epithelial cells and productions of neuropeptides by the inflammatory cells [43, 44, 45]. Two-third (2/3) of the patients sampled had fever. There was a significant ($\chi2$= 5.444; p= 0.019) association between fever and the presence of *Cryptosporidium* copro-antigens, also with a high prevalence in those with fever (19.7%) against those that do not have fever (6.7%). Though this findings contradicts the reports of El-Helaly [26] and Nyamwange [15] which showed no significant association between cryptosporidiosis and fever, but reports of Okojokwu [27] supports our findings. The fever may be due to secretion of cytokines, interleukin-1 (IL-1) by the host cells in response to the response presence of the parasite which induces fever. The observation made from this study showed insignificant association ($\chi2$= 4.326; p= 0.228) between the detection of *Cryptosporidium* antigens and source of water. Though the highest prevalence was observed in patients that use stream water, which may be due to the contamination by man and animal excreta as seen in the study area. There was a significant ($\chi2$= 6.685; p= 0.0097) between consumption of raw vegetables and cryptosporidiosis, this may be as a result of contamination of the water source to this vegetables. Also, no significant association was observed in animal contact ($\chi2$= 1.503; p= 0.220), toilet system ($\chi2$= 5.049; p= 0.080) and cryptosporidiosis.

## CONCLUSION

The findings in this study indicates that *Cryptosporidium* copro-antigens is prevalent in the study population. Children under 5 years of age had higher prevalence of cryptosporidiosis than older patients. Symptoms including abdominal pain, fever and diarrhea were found to be associated with cryptosporidiosis. Among the risk factors studied, consumption of raw vegetables was significantly associated with *Cryptosporidium* infection. It is therefore pertinent that contacts with animals and their dung be minimized. Water should be boiled or filtered before drinking. Further studies are recommended to determine the molecular profile of the different species and genotypes of *Cryptosporidium* in circulation in Sokoto State, Northwestern Nigeria.

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