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# Serological and molecular characterization of *Cryptosporidium* species from humans in Sokoto State, Nigeria

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**Abstract**

*Cryptosporidium* species are one of the most common causes of gastrointestinal infection in humans around the world. This study aimed at the characterization of *Cryptosporidium* species in humans using the 18S rRNA gene. Among the 368 human faecal samples screened using *Cryptosporidium* antigen Copro-ELISA kit, 61 (16.6%) were positive. The positive faecal samples were subjected to Nested PCR for the amplification of 830 bp fragments of small subunit (SSU) rRNA gene and followed by nucleotide sequencing. Out of the 61 copro-ELISA positive samples, 5 (8.2%) were PCR positive for *Cryptosporidium* species (3 (4.9%) of *C. parvum* and 2 (3.3%) of *C. hominis*). Two HIV patients were found to be harbouring *C. parvum* and *C. hominis*, so also as hypertensive and diarrheic patients harbouring *C. parvum* and *C. hominis,* respectively. Higher prevalence rates of *Cryptosporidium* was found in young children (11.1%), males (8.7%), loose faeces (42.9%) than older age groups (8.7%), females (7.9%) and well-formed (3.1%) or mucoid/pasty faeces (0%) based on the data gathered from the close-ended questionnaire also used on each human subject. This study was the first to report *C. parvum* and *C. hominis* infecting humans in Sokoto state, Northwestern Nigeria. It is suggested that a multi-locus study of *Cryptosporidium* species in developing countries would be necessary to determine the extent of transmission of *Cryptosporidium* in the populations.

*Keywords*: 18S gene, *Cryptosporidium species*, Humans, Molecular characterization, Serology, Sokoto, SSU gene

## Introduction

*Cryptosporidiosis* is a parasitic zoonotic disease affecting all land and most aquatic animals (Bamaiyi & Redhuan, 2016). It is caused by many species of the genus *Cryptosporidium* and ranks 5th among the 24 most important food-borne parasites worldwide (Aniesona & Bamaiyi, 2014). *Cryptosporidium spp* are

obligate intracellular parasites of humans and animals with more than 40 genotypes which are reported to have zoonotic potential (Bamaiyi & Redhuan, 2016; Koehler *et al.,* 2018). *Cryptosporidium* species (*spp*.) are considered one of the most important parasitic diarrheal agents worldwide (Bodger *et al.,* 2015). Humans and animals get infected when they consume food and drink containing oocysts of these protozoan parasites. Due to the food and water transmission, the incidence and prevalence of *Cryptosporidiosis* are higher in less developed and developing countries where people are insufficient of basic infrastructure or fundamental facilities to avoid food and drinking water contaminated by infectious oocysts in faeces (Burnet *et al.,* 2014). The disease usually manifests as a self-limiting or progressively life-threatening diarrhoea in immune-competent individuals and immune-compromised patients like HIV/AIDS patients, the young and the elderly; individuals undergoing cancer chemotherapy, and any other condition that compromises the immune system including simple malnutrition. This parasite is responsible for the morbidity and mortality of millions of individuals every year (Verweij *et al.,* 2004). Watery diarrhoea, abdominal cramps, nausea, anorexia and low-grade fever are the most commonly observed symptoms but fatal diarrhoea with malabsorption and dehydration occurs in young and immune-compromised hosts (Mehendra *et al.,* 2021). *Cryptosporidium* infection can have devastating effects on people that wear contact lenses as it may cause problems such as conjunctivitis, blepharitis and keratitis when the lenses are washed in contaminated water (Anon, 2007). *Cryptosporidium* is resistant to chlorine and other disinfectants used in water treatment. Because of their small size, conventional municipal water treatment filters cannot easily remove them from water (Hargy & Clancy, 2008). Resistance of this parasite to water treatment makes many people unaware of the parasitic infestation and therefore do not seek medical attention or are not tested for this parasite and so *Cryptosporidium* often goes undetected as the cause of an intestinal illness (Avery *et al.,* 2007). There are five human pathogenic *Cryptosporidium* species *(C. hominis, C. parvum, C. meleagridis, C. felis and C. canis)* and they have different spectrum of host specificity, therefore characterisation of *Cryptosporidium* at the species level will be useful in identifying infection and contamination sources (Xiao *et al.,* 2004). Detection of *Cryptosporidiosis* using PCR-based methods is more sensitive than by conventional microscopical and serological methods for detecting oocysts in faeces. Molecular methods can also identify the species/genotypes and subtypes of *Cryptosporidium*, important for determining the epidemiology of *Cryptosporidium* and predicting transmission routes (Caccio, 2005). Since the description of the first PCR-based tool for the differentiation of *C. parvum* and *C. hominis*, molecular techniques in the diagnosis of *Cryptosporidiosis* became popular, especially due to their genotyping capabilities. This study was conducted to identify the *Cryptosporidium* species found in humans in Sokoto State, Northwestern Nigeria using the Nested-PCR technique and sequencing.

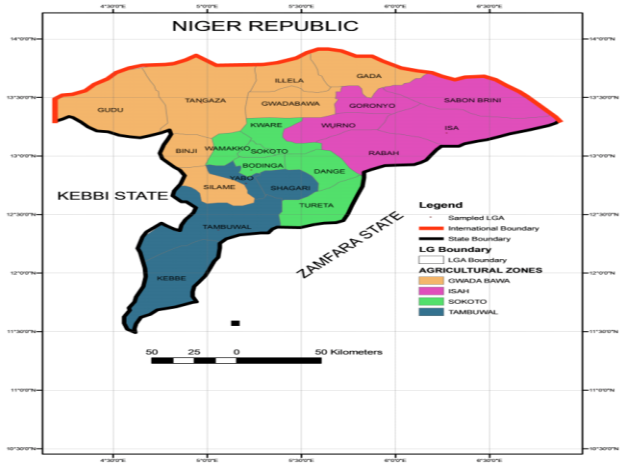


**Figure 1**: Sokoto State map showing the four Agricultural zones of the state
Source: Sokoto State Ministry of Animal Health and Fisheries Development

## Materials and Methods

*Study design*

This study was a prospective cross-sectional, hospital-based study carried out between December 2015 and October 2016.

*Study area*

Sokoto State lies on latitude 13°N and between longitudes 4.8°E and 6.54°E in Northwestern Nigeria. The state shares a border with Niger Republic to the north, Kebbi State to the south, and Zamfara State to the east. Based on the 2006 census, Sokoto State has an estimated population of 4,344,399. (Blench, 1999). There is one general hospital in each of the 23 LGAs within the four zones. One LGA is selected from each of the four zones by balloting; and they are Wurno LGA (Isah zone), Tambuwal LGA (Tambuwal zone), Tureta LGA (Sokoto zone) and Gwadabawa LGA (Gwadabawa zone) (Figure 1).

*Target population*

The patients studied attended the sampled Local Government general hospitals because of varying illnesses such as gastroenteritis, tuberculosis, hypertension, helminthosis, malaria, typhoid fever and HIV/AIDS. Those with gastroenteritis had either accompanying diarrhoea or abdominal pain. Those with malaria and typhoid fever had either accompanying gastroenteritis or abdominal pain.

The study was carried out considering patients presented with diarrhoea, immunocompromised conditions such as HIV, Hepatitis, and children ≤ 5 years of age, all consented to take part in the study. Before stool sample collection, ethical clearance was sought and issued by the Ethical Committee of Sokoto State Ministry of Health, Nigeria (SMH/1285/VOL.III/03). Samples were then collected from patients who attended the four General Hospitals of each of the selected Local Government Areas of the four Agricultural zones of the State, namely: Wurno, Yabo, Gwadabawa General Hospitals and Sokoto Specialist Hospital, Sokoto State.

*Inclusion criteria*

All patients who were presented with diarrhoea, immunocompromised individuals or children ≤ 5 years of age (parents of the children) in the hospital gave their consent and assent respectively to participate in the study.

*Exclusion criteria*

All non-diarrheic patients, immunocompetent individuals or children > 5 years of age presented in the hospital during the study were not considered for sample collection.

*Questionnaire*

A structured questionnaire was used to collect demographic data and patients' information on age, gender, feeding, water source, educational background, sanitation and symptoms.

*Sample collection*

Three hundred and sixty-eight (368) freshly voided faeces were collected in a wide-mouthed sample container. Ten percent 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 analysed. The molecular analyses of the Copro-ELISA positive samples were carried out at DNA Labs Limited, RC: 1027690, Q5 Danja Road, Off Katuru Road, Unguwar Sarki, Kaduna State, Nigeria.

*Detection of Cryptosporidium copro-antigens by copro-ELISA*

The detection of *Cryptosporidium* species coproantigens in the samples was performed using a commercially available Copro-ELISA kit for faecal samples (*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 instructions. 0.1g of each faecal sample was homogenised 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), and 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 wells and read using the ELISA reader (BIOTEC; Model: ELx800, Biotex Instruments, USA) at 450/620nm.

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

A Nested Polymerase Chain Reaction (SSU rRNA PCR) and DNA sequencing was used in analysing all Copro-ELISA positive samples.

*DNA extraction and purification*

Stool DNA Extraction Kit (E.Z.N.A®) was used for the extraction of DNA in faecal samples according to manufacturers' instructions. The tubes were centrifuged at a maximum speed (3500 rpm) for 1 minute and the DNA aliquot was stored at -20$^0$C.

*Nested-PCR amplification of SSU rRNA (18S and GP60) gene locus*

All amplifications were performed using a PTC-100 Programmable Thermal Controller (Peltier-Effect Cycling, MJ Research Inc., Ohio, USA). A highly polymorphic section of the SSU rRNA gene was amplified by Nested PCR as described by Xiao *et al.* (1999a). The method involved the amplification of an approximately 1325bp-long primary product followed by a secondary amplification of an internal fragment with a length of approximately 830bp. The fragment has been shown to be highly specific for

species and genotype identification of *Cryptosporidium.*

Initial amplification was performed in a 20 µl volume containing 1 µl of DNA template, 15 µl of distilled water, 1 µl of Crypto I (Forward, F1) Primer (FI: LX0697): '5-TTCTAGAGCTAATACATGCG-3' (33.8nm/0.21mg), 1 µl of Crypto II (Reverse, R1) Primer (R1: LX0669): '5-TGATCCTTCTGCAGGTTCACCTACG-3' (24.9nm/0.16mg) dissolved in a lyophilised blue pellet of AccuPower® HotStart PCR Premix (Catalog K-5050[a]) consisting of 1unit of HotStart DNA polymerase, 1 unit of PCR buffer, 250 µM of dNTP, 1.5mM $MgCl_2$.

The optimized initial amplification conditions were as follows: $95^0$C: for 5 minutes (Pre-denaturation): 35 cycles of amplification: $94^0$C for 45 seconds, $60^0$C for 45 cycles (Annealing) and $72^0$C for 60 seconds (Extension). The final extension step lasted for 10 minutes at $72^0$C and the cooling (soaking) temperature was $4^0$C.

The optimised conditions for second-round PCR were the same as those for the first round, except that 3 µl of the Primary was used for DNA amplification product and the primers used were 1 µl of Crypto III (Forward, F2) Primer (F2:LX0698): 5'-GGAAGGGTTGTATTTATTAGATAAAG-3' (22.3nm/0.18mg), 1 µl of Crypto IV (Reverse, R2) Primer (R2: LX0670): 5'-AAGGAGTAAGGAACAACCTCCA-3' (27nm/0.18mg). Each set of experiments included a Positive PCR control consisting of 1 µl of specific DNA template (*C. parvum* was used in this case) and a Negative PCR Control was the master mix without any *Cryptosporidium* DNA.

*Agarose gel electrophoresis*

Amplifications were confirmed by 1.5% agarose gel electrophoresis and DNA fragments visualised on an ultraviolet trans illuminator (GEL, DOC 2000 BIO-RAD).

*Nucleotide sequencing*

The GP60 glycoprotein from human samples were sequenced using a Beckman Coulter, CEQ™ 2000XL DNA Analysis System (Beckman Coulter Inc., Bioresearch Division, Palo Alto, CA).

*Secondary PCR for sequencing*

The positive reactions that showed bands at the expected size under the UV transilluminator were selected for sequencing. The amplified DNA from secondary PCR products was separated by gel electrophoresis and sent for sequencing using a Beckman Coulter CEQ™ 2000XL DNA Analysis System (Beckman Coulter Inc., Bioresearch Division, Palo Alto, CA).

*Editing and aligning sequence data*

All sequences were assembled, forward and reverse checked for accuracy. Chromaspro® software was opened and sequences were imported and areas to be read were defined. The peaks against the base call were checked and 5'-3' end limits were determined all sequences were assembled to generate a contig file and the files were saved as gpj after checking peaks and base call. The sequence was exported to the editor (Blast search) and the species which corresponded with each sequence was determined and it was checked for plus (forward) and minus (reverse) signs. Forward sequences were saved as fasta files, while reverse sequences were reversed to plus sign before saving as fasta files. Sequences were then aligned by opening fasta files with Notepad for the creation of single text files.

*Sequence analysis*

All the sequences were subjected to a BLAST search to determine their identities and assess their homologies and similarities to those in GenBank. Electropherograms were generated by the sequencer and were `read out using the ChromasPro™ software.GP60 sequences of *Cryptosporidium* species generated were aligned with each other and reference the sequences using the software ClustalX. Sequence alignment was checked for sequencing accuracy using the software BioEdit. The electropherograms were re-checked for any sequence uncertainty and subtype designation was determined based on the sequence identity to reference sequences and the number of trinucleotide repeats using the subtype nomenclature described by Sulaiman *et al.* (2005).

*Statistical analysis*

The data obtained were computed and analysed using IBM Statistical Package for Social Sciences (SPSS) Version 23 (SPSS Inc., USA) at a 95% confidence level. Data on the samples examined and as well as positive ones were summarised and presented as frequency and percentages on 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% C. I) were calculated to measure the strength of association between variables and the occurrence of *Cryptosporidium* oocysts. $p$ – values ≤ 0.05 were considered significant.

**Results**

Gastroenteritis/diarrhoea patients (23.3%) had the highest prevalence of *Cryptosporidium* antigen among all the disease sample specimens screened using Copro-ELISA, followed by HIV/AIDS (15.8%) patients, helminthosis (15.2%) patients, tuberculosis (14.3%) patients, malaria/typhoid fever (12.7%) patients, hypertensive (12.1%) patients and the least of all were malnourished patients with 7.1% prevalence (Table 1).

DNA preparations of all the 61 Copro-ELISA *Cryptosporidium* positive faecal specimens showed that 5 (8.2%) yielded products of the expected size (830 bp) in the Nested PCR analysis of the SSU rRNA gene (Table 2). Yabo Local Government Area (LGA) was found to have a higher prevalence (16.7%), followed by Wurno (10.3%), Sokoto (7.1%) and Gwadabawa had zero prevalence (Table 2).

DNA sequencing of the secondary SSU rRNA PCR products showed three (4.92%) out of the five to be *C. parvum*, while the remaining two (3.28%) of the samples were *C. hominis*.

Two (11.1%) out of the 18 (29.5%) humans that were two years or younger were positive for *Cryptosporidium*, followed by two (8.7%) out of the 23 (37.7%) patients within 21 to 60 years were positive and 1 (9.1%) out of the 11 (18.0%) patient within 60 to 70 years was positive also. Although, no *Cryptosporidium* was seen in patients between 2 to 20 years old (Table 3).

Table 4 shows the relationship between *Cryptosporidium* and sex, contact with animals and consumption of raw vegetables. Out of the 23 (37.7%) male faeces examined, 2 (8.7%) were PCR positive for *Cryptosporidium*, while out of the 38 (62.3%) female faeces examined, 3 (7.9%) were PCR positive.

Three (7.9%) of the 39 (64.0%) faeces from patients that had contact with various animal species were PCR positive for *Cryptosporidium*, while 2 (9.1%) of the 22 (36.0%) faeces of patients that had no contact with animals were PCR positive (Table 4).

Two (4.4%) out of the 45 (73.8%) faeces from patients that consume raw vegetables were PCR positive for *Cryptosporidium*, while 3 (18.8%) out of the 16 (26.2%) samples from patients that do not consume raw vegetables were PCR positive (Table 4). Among the 7 (11.5%) loose faeces examined, 3 (42.9%) were

**Table 1**: Prevalence of *Cryptosporidium* according to disease/conditions in human patients in Sokoto State using Copro-ELISA positive samples

| Disease/Condition | No. examined | Positive samples | Specific rate (%) |
|---|---|---|---|
| Hypertension | 33 | 4 | 12.1 |
| HIV/AIDS | 76 | 12 | 15.8 |
| Malnutrition | 28 | 2 | 7.1 |
| GIT/Diarrhoea | 116 | 27 | 23.3 |
| Malaria/Typhoid | 55 | 7 | 12.7 |
| Helminthosis | 46 | 7 | 15.2 |
| Tuberculosis | 14 | 2 | 14.3 |
| Total | 368 | 61 | 16.6 |

**Table 2**: Prevalence of *Cryptosporidium* found in human patients using SSU rRNA PCR from Copro-ELISA positive samples conducted in LGA's of Sokoto State

| LGA's | Number of faecal samples examined (%) | No. and prevalence of positive samples (%) |
|---|---|---|
| Yabo | 6 (9.8) | 1 (16.7) |
| Sokoto | 14 (23) | 1 (7.1) |
| Wurno | 29 (47.5) | 3 (10.3) |
| Gwadabawa | 12 (19.7) | 0 (0) |
| Total | 61 (100.0) | 5 (8.2) |

**Table 3**: Prevalence of *Cryptosporidium* found in human patients in relation to their ages in Sokoto State

| Age (Years) | No. examined | Positive samples | Specific rate (%) |
|---|---|---|---|
| 0 - 2 | 18 | 2 | 11.1 |
| 3 - 20 | 9 | 0 | 0.0 |
| 21 - 60 | 23 | 2 | 8.7 |
| 61 - 70 | 11 | 1 | 9.1 |
| Total | 61 | 5 | 8.2 |

**Table 4**: Some Socio-demographic factors associated with the prevalence of *Cryptosporidium* species identified in Sokoto State

| Factors | No. examined | Positive samples | Specific rate (%) |
|---|---|---|---|
| Sex | | | |
| Male | 23 | 2 | 8.7 |
| Female | 38 | 3 | 7.9 |
| Contact with Animals | | | |
| Yes | 39 | 3 | 7.9 |
| No | 22 | 2 | 9.1 |
| Consumption of Raw Vegetables | | | |
| Yes | 45 | 2 | 4.4 |
| No | 16 | 3 | 18.8 |

**Table 5**: Prevalence of *Cryptosporidium* according to faecal consistency in humans in Sokoto State, Northwestern Nigeria using SSU rRNA PCR

| Faecal Consistency | No. examined | Positive samples (%) | Specific rate (%) | Chi-Square ($x^2$) | P-value |
|---|---|---|---|---|---|
| Loose | 7 | 3 | 42.9 | 9.30 | 0.02 |
| Watery | 9 | 1 | 11.1 | df=3 | |
| Well-formed | 32 | 1 | 3.1 | | |
| Mucoid/Pasty | 13 | 0 | 0 | | |
| **Total** | 61 | 5 | | | |

**Table 6**: Prevalence of *Cryptosporidium* species according to the source of water in humans in Sokoto State using SSU rRNA PCR

| Source of water samples | No. examined | Positive | Specific rate (%) |
|---|---|---|---|
| Well only | 7 | 0 | 0 |
| Borehole only | 4 | 0 | 0 |
| Packaged/Tap water | 0 | 0 | 0 |
| None | 9 | 2 | 22.2 |
| Borehole/well water | 16 | 1 | 6.25 |
| Borehole/Packaged/Tap | 2 | 0 | 0 |
| Borehole/Well/Packaged | 3 | 0 | 0 |
| River only | 20 | 2 | 10 |
| Total | 61 | 5 | 8.2 |

positive for *Cryptosporidium*, 1 (11.1%) out of the 9 (14.8%) watery faeces examined was PCR positive and also 1 (3.1%) out of the 32 (52.5%) well-formed faeces examined was PCR positive for *Cryptosporidium*. None of the mucoid/pasty faeces examined was PCR positive (Table 5).

There was a higher prevalence (22.2%) of *Cryptosporidium* in patients that did not drink water from any source but were on exclusive breastfeeding, followed by those that drank from the river only (10%) and those of borehole/well (6.25%). No *Cryptosporidium* was discovered from all other sources of water (Table 6).

The two *C. hominis* were recovered from an 18-month-old diarrheic female child and a 380-month-old HIV-positive male. Their sources of water are rivers and wells. The 380 months (32 years) old HIV patient has contact with cattle and sheep and also eats raw vegetables. While the three *C. parvum* were detected in the faeces of a 15 months old malnourished female child, who only drank from the mother's breast milk and was also found in the faeces of 408 month (34 years) old HIV male patient, who has contact with chickens and drank from borehole and well water. *C. parvum* was also discovered in the faeces of 744 (62 years) months old hypertensive female patients, who had contact with goats and chickens (Table 7).

Results of this study further suggest that the traditional screening or detection methods (Microscopy, Ziehl-Nelson staining etc.) might lead to low detection of *Cryptosporidium* species with low oocysts shedding intensity compared to immunoassays and molecular techniques that are highly specific and sensitive to the parasite.

The molecular findings in this study show that 1.5% ethidium bromide-stained agarose gel shows DNA amplified at small subunit (SSU) rRNA gene from

**Table 7**: Summary of species of *Cryptosporidium* identified in faeces of human patients in Sokoto, Northwestern Nigeria

| Age (Yrs) | Sex | Type of Stool | Condition | Location | Eat raw vegetables | Contact with animals | Species identified |
|---|---|---|---|---|---|---|---|
| 1.5 | F | Loose | Diarrhoea | Wurno | No | No | *C. hominis* |
| 1.3 | F | Loose | Malnutrition | Yabo | No | No | *C. parvum* |
| 34 | M | Watery | HIV | Sokoto | No | Chickens | *C. parvum* |
| 31.5 | M | Loose | HIV | Wurno | Yes | Cattle, Sheep | *C. hominis* |
| 62 | F | Normal | Hypertension | Wurno | Yes | Goat, Chicken | *C. parvum* |

human faeces in Sokoto state. Lanes 2, 4, 9 and 20 have 830-bp bands positive for *Cryptosporidium spp.* $N_0$ is a master mix negative control, $P_0$ is *C. parvum* positive control. M is 100-bp ladder. Arrow points to 500-bp band (Figure 2a). Similarly, figure 2b indicates 1.5% ethidium bromide-stained agarose gel showing DNA repeatedly amplified at small subunit (SSU) rRNA gene from the identified positive (those samples with bands at 830-bp, Lane 1 - 9) human faeces in Sokoto state for DNA sequencing using their extra-large comb bands (Band cutting). $N_0$ is a master mix negative control, $P_0$ is *C. parvum* positive control M is 100-bp ladder. Arrow points to 500-bp band.

Two *C. hominis* and three *C. parvum* were obtained from the nucleotide sequences. The sequences were aligned with fourteen reference nucleotide sequences obtained from previous CDC studies and GenBank. The obtained *C. parvum* sequence in this study showed 97% identity with reference sequences KF128753.1, KM215743.1 and DQ010953.1 upon BLAST search. The *C. hominis* obtained showed 99% identity to CQ865523.1 and EU03234.1 reference sequence from the GenBank upon BLAST search.

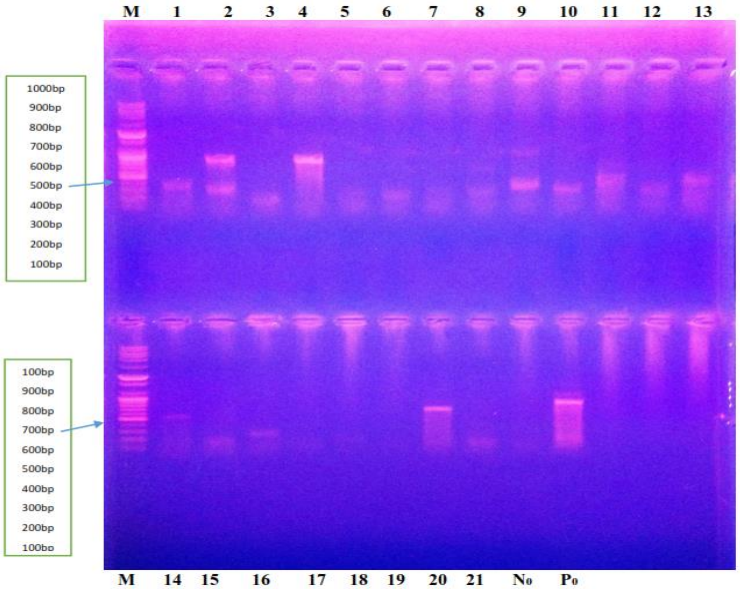


**Figure 2a:** A 1.5% ethidium bromide-stained agarose gel showing DNA amplified at small subunit (SSU) rRNA gene

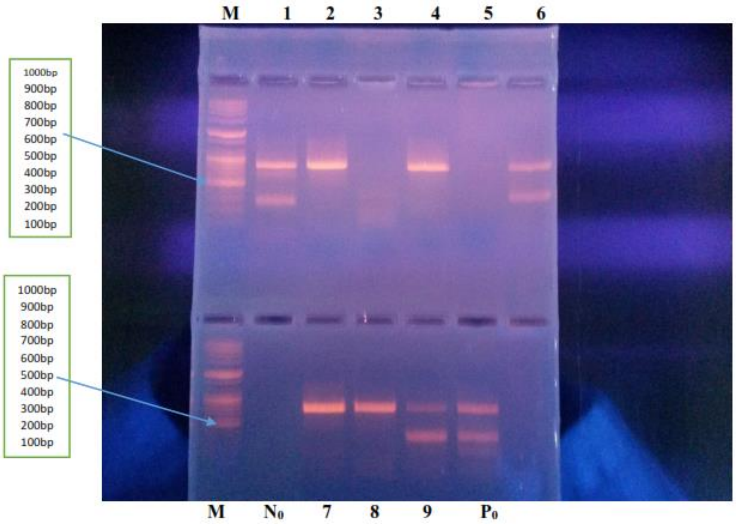


**Figure 2b**: A 1.5% ethidium bromide-stained agarose gel showing DNA repeatedly amplified at small subunit (SSU) rRNA gene

**Discussion**

The prevalence (8.2%) observed in this study is lower than 42.9% reported by Aniesona and Bamaiyi 2014 in Nigeria. This disparity may be a result of the patients sampled in this study being significantly younger than what was used in the other study. Higher prevalence was also observed in children that were younger than two years in this study as reported in Nigeria (Salman and Kalantari, 2012), Iran (Taghipour *et al.,* 2011) and Kuwait (Iqbal *et al.,* 2011).

Results of the study have shown the presence of *C. parvum* (4.9%) and *C. hominis* (3.3%) which are the most common cause of human *Cryptosporidiosis,* especially in industrialised nations (Xiao, 2009). The dominance of *C. parvum* in this study is similar to

some studies conducted in Iran, Malawi, Nigeria, Vietnam and Kuwait (Iqbal *et al.,* 2011: Salman and Kalantari, 2012; Taghipour *et al.,* 2011), but differs with the distribution of *Cryptosporidium* genotypes in Nigeria, Peru, Malawi, Thailand, Uganda, Kenya and South Africa which showed a dominance of *C. hominis* in children and adult (Leav *et al.,* 2002; Gatei *et al.,* 2003; Peng *et al.,* 2003; Maikai *et al.,* 2012). Also, several studies in Europe have shown a slightly higher prevalence of *C. parvum* than *C. hominis* in both immunocompetent and Immunocompromised persons (Guyot *et al.,* 2001; Chalmers *et al.,* 2002; Alves *et al.,* 2001). The differences in the distribution of *Cryptosporidium* genotypes in humans are considered an indication of differences in infection sources (McLaunchlin *et al.,* 2000; Learmonth *et al.,* 2004).

The distribution of *C. parvum* and *C. hominis* in humans differ in geographical regions. In European countries, both *C. parvum* and *C. hominis* are common in humans (Bajer *et al.,* 2008; Savin *et al.,* 2008; Zintl *et al.,* 2009). In the Middle East, *C. parvum* is the dominant species in humans (Sulaiman *et al.,* 2005; Meamar *et al.,* 2007; Tamer *et al.,* 2007; Al-Brikan *et al.,* 2008). In the rest of the world, especially developing countries, *C. hominis* is usually the predominant species in humans (Cordova Paz Soldon *et al.,* 2006; Gatei *et al.,* 2007; Gatei *et al.,* 2008; Samie *et al.,* 2006; Hung *et al.,* 2007; Jex & Gasser, 2008). Geographic variations in the distribution of *C. parvum* and *C. hominis* can also occur within a country. For example, *C. parvum* is more common than *C. hominis* in rural states in the United States and Ireland (Feltus *et al.,* 2006; Zintl *et al.,* 2009).

The higher prevalence of *Cryptosporidium* in the younger (0-24 months) old age group observed in this study agrees with other studies in which *Cryptosporidium* spp are more prevalent in children younger than two years of age (Sulaiman *et al.,* 2005) and peak occurrence of infections and diarrhoea (Bern *et al.,* 2000). The infection is more common and severe in malnourished children (Hunter & Nichols, 2002). This may be attributed to the increased susceptibility of younger children to the infection after the weaning period, they may be exposed to the *Cryptosporidium* oocysts within the environment due to their explorative habit at that stage. Age-associated variations in *C. parvum* and *C. hominis* have been reported by Chalmers *et al.* (2009). In the Netherlands, *C. hominis* was more commonly found in children and *C. parvum* more in adults (Bajer *et al.,* 2008). In the United Kingdom, *C. hominis* was more prevalent in infants less than one year, females aged 15-44 years and international travellers and there has been a decline in the cases of *C. parvum* since 2001 (Chalmers *et al.,* 2008; Chalmers *et al.,* 2009). In a study conducted in Peru, there were no significant differences in the distribution of *Cryptosporidium* species or genotypes between children and HIV-positive persons, from which they concluded that there was no preferential infection with zoonotic species or genotypes in immuno-compromised persons (Cama *et al.,* 2007; Cama *et al.,* 2008). In Nigeria, *C. parvum* and *C. hominis* were isolated in children of 10 months and 6 months of age at Kaduna, Northwestern Nigeria respectively by Maikai *et al.* (2012).

The *Cryptosporidium* infection rate of 8.2% in this study is higher than in studies conducted in Kaduna state, Northwestern, Southeastern and Central Nigeria, where *Cryptosporidium* oocysts were not detected in the stool of 189 HIV-infected and uninfected patients (Nwokediuko *et al.,* 2002) and 52 malnourished HIV-infected children (Banwat *et al.,* 2004). In contrast, higher infection rates of 9-52.7% were reported in other studies in Nigeria (Adesiji *et al.,* 2007; Akinbo *et al.,* 2010; Molloy *et al.,* 2010; Ayinmode *et al.,* 2012). The differences in *Cryptosporidiosis* occurrence may be attributed to differences in patients' populations, geographic locations and detection methods (Zaidah *et al.,* 2008). In addition, the prevalence of *Cryptosporidium* is known to vary from region to region, because of differences in infection sources, the extent of environmental contamination and other risk factors associated with the acquisition of infections (Adesiji *et al.,* 2007).

The higher prevalence of *Cryptosporidium* species seen in males than females is similar to the previous reports of Maikai *et al.* (2012) and Ayinmode *et al.* (2012). In this study, only patients that did not have contact with animals had a higher prevalence of *Cryptosporidiosis* than those that had contact, this may be because there are several other sources by which humans can acquire the infection. Contact is one of them, but if the animal involved is not infected, then the transmission will not occur even if there is contact between the animal and man. Moreover, the potential host ranges and transmission pathways of the potentially zoonotic species to humans are yet to be documented, as having pets does not appear to be a risk factor (Fayer *et al.,* 2000). Two of the patients who were identified to be infected with *Cryptosporidium* in

this study were less than 2 years as such it may be obtained by milk bottle contamination or un-breast feeders and creeping on the contaminated ground (Rahi *et al.,* 2013).

Patients that did not eat raw vegetables had a higher prevalence of *Cryptosporidium* oocysts detected in their faeces than those that ate, probably because the vegetables are eaten raw were not contaminated with the oocysts and there may be far more important ways of acquiring the infection within the study area (Robertson and Gjerde 2001). Other ways rather than consumption of raw vegetables include: raw consumption of crops can enhance the chance of transmission of intestinal parasites and uncontrolled use of treated or untreated wastewater can enhance the risk of spreading parasites to vegetable farms (Ali *et al.,* 2019); the presence of viable oocyst of *Cryptosporidium* in final effluent of wastewater treatment plant (Gennaccaro *et al.,* 2003; Ajonna *et al.,* 2012; Alonso *et al.,* 2014) and Seafood (Freire-Santos *et al.,* 2000).

It is known that *Cryptosporidium* infection in animals and humans produces different levels of infectivity and severity and even different responses to treatment (Caccio *et al.,* 2002). Thus, it is not surprising that there was a higher prevalence in patients with loose faeces as *Cryptosporidium* species recovered from them may be associated with diarrhoea or there may be some underlining factors that may have worsened the condition.

A prevalence of 8.2% using PCR in human faeces from *Cryptosporidium* Copro-ELISA positive samples was seen in this study, probably because there were relatively low *Cryptosporidium* oocysts count in some of the samples, also the presence of PCR inhibitors in the faecal samples such as bile acids, haemoglobin and complex-polysaccharides even though they are present at low concentration can affect the prevalence, extraction procedures and failure to remove sample preservatives and fixatives and oocysts age may also affect the prevalence (Iqbal *et al.,* 2011), failure of cell lysis, nucleic acid degradation and capture of an insufficient amount DNA (Salman and Kalantari, 2012) can also contribute to the low prevalence. It may also be because of the drying up of some of the samples as the specimens returned by some of the patients were very small. The findings of this study further suggest that the traditional screening or detection methods (Microscopy, Ziehl-Nelson staining etc.) might lead to low detection of *Cryptosporidium* species with low oocysts shedding intensity compared to immunoassays and molecular techniques that are highly specific and sensitive to the parasite.

The result of this study demonstrated the significance of *Cryptosporidium* and sources of drinking water as packaged water which is believed to be more purified had no prevalence, while those that drank no water had a higher prevalence. The explanation here was that maybe there was a milk bottle contamination and roaming around in an infected environment (Rahi *et al.,* 2013). Water-borne transmission of *Cryptosporidium* is a significant difference in children in tropical countries (Bern *et al.,* 2000). *Cryptosporidium parvum* and *C. hominis* are associated with most waterborne, foodborne and direct contact-associated (person-to-person and animal-to-person) outbreaks of *Cryptosporidiosis* (Bern *et al.,* 2000). The *C. parvum* found in this study may not indicate a zoonotic transmission as not all *C. parvum* infections in humans are as a result of zoonotic transmission (Alves *et al.,* 2003; Mallon *et al.,* 2003; Xiao *et al.,* 2004b), though it may indicate that anthroponotic transmission of *C. parvum* occurs in Sokoto State. This is further justified by the relatively higher number of *C. hominis* detected, suggesting further the importance of anthroponotic transmission of *Cryptosporidiosis* in the area. *C. parvum* is commonly seen in both humans and ruminants in many areas and in most areas studied (Alves *et al.,* 2003; Peng *et al.,* 2003).

In conclusion, the present study has shown that humans in Sokoto State were infected with *Cryptosporidium.* Though it is known that *Cryptosporidium* infection predisposes humans to other enteric pathogens, none of the individuals tested was infected with more than one species of *Cryptosporidium* as there was no copathogen infection between them. But it is possible for an individual to be infected with different genotypes of the same species.

*Cryptosporidium hominis* and *C. parvum* were the two species detected in humans. The study has contributed to a deeper understanding of the species of *Cryptosporidium* in Sokoto state, Northwestern Nigeria. The epidemiologic data collected in this study failed to identify the risk factors associated with the acquisition of the infections. This may be due to the information gathered from the questionnaire.

The findings in this study may help us in mounting control and prevention strategies against *Cryptosporidiosis* in humans.

The Copro-ELISA technique employed in this study, although reported to be highly sensitive and specific in the detection of *Cryptosporidium* species has the risk of producing false positives as seen in some of the Copro-ELISA positive results in this study. As such utilising molecular technique to detect and characterise the *Cryptosporidium* species found in humans in this study is very important and the only option to identify the parasite up to species level as it was reported to be highly specific and sensitive when compared to ELISA and microscopy.

Multi-locus sequence typing (MLST) of *Cryptosporidium* species from this geographic region needs to be conducted to determine the extent of transmission of *Cryptosporidiosis* within the population.

A wider study involving various age groups should be carried out to have a better understanding of genotypes from these hosts.

In the future, a wider study involving more respondents should be conducted to formulate a reliable estimate of the disease burden in the country. Apart from information on age, sex, contact with animals, sources of water, consumption of raw vegetables and faecal consistency, other variables could be included such as symptoms, current chemotherapeutic regimen being taken by the respondents, socioeconomic status and hygienic practices of households.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.

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