



Research Article

Occurrence of *Cryptosporidiosis* in Vended Sachet Water and Sewage in Maiduguri, Borno State, Nigeria

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ABSTRACT

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Cryptosporidium is a small protozoan parasite that infects the microvillus region of epithelial cells in the digestive and respiratory tract of vertebrates. It is an obligate intracellular parasite of man and other mammals, birds, reptiles and fish. It requires its host to multiply. This study examines the occurrence of one of the most frequent aquatic human parasite protozoa *Cryptosporidium* species. Infected humans, livestock, and other animals shed vast numbers of *Cryptosporidium* oocysts into the environment, which signify the resistant life cycle stage. Sludge, raw sewage, sewage effluents, drinking water, and recreational waterways all encompass these oocysts. A total of 59 vended sachet water and sewage samples were collected from various locations in Maiduguri metropolis for the detection of *Cryptosporidium* spp. Out of the 59 samples collected, 51(86.4%) are sachet water from various table water companies and 8(13.6%) sewage samples from different locations in the city. Modified Ziehl Nelson Staining technique was used in staining the samples. The stained slides were examined using 40× and higher objective 100 × oil immersion objectives to confirm the morphology of the oocysts. Out of the 51(86.4%) vended water analyzed, no oocyst was detected. However, 3 (37.5%) were detected from sewage at different locations in the metropolis. It was indicated that there was proper handling and good hygiene practice during processes of the vended sachet water from their manufacturers; it is recommended that proper disposal of refuse should be advocated in the study area and sewage needs to be adequately treated before being recycled for use.

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INTRODUCTION

One of the most frequent aquatic human parasite protozoa is *Cryptosporidium parvum*. It usually infect humans, livestock, and other animals which are shed vast numbers of their oocysts into the environment. Sludge, raw sewage, sewage effluents, drinking water, and recreational waterways all contain these oocysts (Bukhari *et al.* 1997; and Fayer *et al.* 2000; Robertson *et al.* 2000; and Caccio` *et al.* 2003), and may represent a public health risk in developed and developing countries (Le Chevalier *et al.* 1991). Numerous watery cryptosporidiosis outbreaks have been detected in different countries in recent years (Fayer *et al.* 2000). They have also been discovered to be highly environmentally persistent, resisting many of the disinfectants typically employed in water treatment plants to prevent bacterial contamination, and the infectious dose has been estimated to be as little as 10 oocysts (Fayer *et al.* 2000).

In industrialized countries, domesticated water is being utilized as an alternative non potable water source for domestic, industrial and agriculture purposes, for which the use of water with low levels of chemical (aluminium, sulfites, nitrates) or microbiological (bacterial, viruses, fungi, protozoan) contaminants would not represent a risk to public or human health (Asano and Cotruvo 2004). Microbiological and parasitological standards, alongside monitoring contamination and the presence of microbial pathogens, are necessary to estimate the risk of infection resulting from exposure to reclaimed and river water (Caccio` *et al.* 2003; and Asano and Cotruvo 2004).

A number of studies about *Cryptosporidium* occurrence in aquatic environments have been reported in the last years (Bukhari *et al.* 1997; Robertson *et al.* 2000; and Caccio` *et al.* 2003), and considerable caution is necessary when extrapolating data from these studies (Bukhari *et al.* 1997). In fact, analysis and comparison of data is difficult because of discrepancies in sampling and concentration procedures and differences in the

epidemiological status of the population. In addition, the procedures for detection and identification of this organism in sewage and water are complex, time consuming, expensive and the oocyst recovery efficiency may be low due to the nature of the specimen. Thus, many samples reported as *Cryptosporidium* might be false negatives. A number of studies have examined the occurrence of *Cryptosporidium* oocysts in sewage and the results are highly variable. Reported densities can range from 4-13700 oocysts per litre in the USA, 2-800 oocysts per litre in the UK and 2-277 oocysts per litre in Italy (Madore *et al.* 1987; and Bukhari *et al.* 1997; and Caccio` *et al.* 2003). *Cryptosporidium* oocysts have been found in reclaimed water at levels of <0.1-15 oocysts per 100 l (Gennaccaro *et al.* 2003; and Quintero-Betancourt *et al.* 2003). Currently employed techniques may not be sensitive enough to analyse the presence of *Cryptosporidium* oocysts in aquatic environments, consequently oocyst detection will require methodological development and standardization in order to establish accurate risk assessment estimations of *Cryptosporidium* oocysts in natural waters. As reported by Asano and Cotruvo 2004, regulations relating to the presence of *Cryptosporidium* oocysts in drinking or recreational waters are available in some states of the USA, Australia, Canada and UK. There are no regulations in mainland European countries for the presence of *Cryptosporidium* oocysts in natural or reclaimed waters, in spite of numerous studies indicating the importance of regulating their presence in natural waters (Rose 1997; Kahana and Tal 2000; Caccio` *et al.* 2003; and Asano and Cotruvo 2004).

Cryptosporidiosis is a gastrointestinal disorder caused by *Cryptosporidium specie*. Human infectivity and clinical outcome data from feeding of adult volunteers support the identification of *C. parvum*, *C. hominis* (the most commonly found species in human worldwide), and *C. meleagridis* as human pathogens (Robinson *et al.*,

1979). *C. cuniculus* (formerly the rabbit genotype) caused a drinking waterborne outbreak in the United Kingdom (UK) (Chalmers *et al.*, 2009), and In a Peruvian shanty village, *C. felis* and *C. canis* were linked to diarrhoea in children Chappel *et al.*, 1999). Small numbers (10) of parasites consumed can induce sickness, according to dose response studies comparing some *C. parvum* isolates to *C. hominis* (Chappel *et al.*, 2006). Hence, with this prevalence, necessitate the study on vended sachet water and sewage in Maiduguri, Borno state.

MATERIALS AND METHODS

Study Area

The study was conducted in Maiduguri metropolis of Borno State which is situated in the Northeastern part of Nigeria. The State lies between latitude 10° North and 13° East and longitude 12° North and 15° East. The state has a total area of 69,436 square kilometers, with a population of 4,098.391 according to 2006 census report (www.borno.state.gov.org). It occupies the greatest part of the Chad Basin. The state has two vegetation zones viz: Sahel in the North which is hot and with severe desert encroachment covering most of the Chad Basin areas and Sudan Savannah in the South which is slightly milder. The rainy season normally begins from June- September in the North and May-October in the South with relative humidity of 49%. Maiduguri is a cosmopolitan town which is inhabited by various ethnic groups but the most dominant are the Kanuri (www.borno.state.gov.org). Borno State shares boundaries with the Republic of Niger to the North, Chad Republic to the North-east and Cameroon to the East. Within the country, the state shares border with Adamawa to the south, Yobe to the West, Bauchi and Gombe to the South-west (Ojo, 1994).

Ethical Consideration

Ethical letter was sort for from the ethical committee of the Borno state Ministry of Environment for clearance and was granted.

Informed Consent

The manufacturers of vended sachet waters were requested for written informed authorization. The researcher kept all information collected for this study private. Furthermore, acceptable data handling practices was rigorously followed. Laboratory coding was utilized to identify the samples collected.

Sample Collection

Sewage samples was collected in sterile plastic universal bottle with a screw cap and labeled accordingly from different locations in the metropolis which include Abbaganaram, Budum round about, Maduganari bayan tudu, Kasuwan shanu abattoir, Blind Workshop Bridge, Bulabulin gidan zana, Bulunkutu bayan makabarta and Gomboru Market Bridge. While vended sachet water was obtained from different companies and transported to the laboratory for analysis.

Sampling Techniques

Purposive non probability sample technique was used to collect sewage sample while systematic random sample technique was used to select vended sachet water companies from which the sample was collected.

Materials

Cover slip, glass slide, Centrifuge Machine, Centrifuge tube, Olympus Binocular Microscope, Pasteur pipette, Absolute methanol, 1% Acid alcohol, kiyoun's carbol fuschin, 3% malachite green. Staining rack.

Procedure

Modified Ziehl Neelsen Staining technique was used. The specimen was centrifuged at 3000g/min for 5minutes, the supernatant was aseptically decanted and 2 drops from the deposit was smeared on a clean grease free glass slide, it was dried on a slide warmer at 60°C until it was completely dried. It was then fixed with absolute methanol for 30 seconds; the fixed slide was stained with Kinyoun's carbol-fuchsin for 10 minute and was rinsed briefly with distilled water and drained. It was decolorized with 1% acid alcohol for 2 minutes and was rinsed with distilled water

and drained. It was then counterstained with Malachite green for 2 minutes and was rinsed briefly with distilled water and drained. It was dried on a slide warmer at 60°C for about 5 minutes. The slide was then examined using 40× and higher objective 100× oil immersion objective.

Data Analysis

Results that were obtained were analyzed using Chi-square test software statistical package for Social Science (SPSS). A Chi-square test was used to compare the differences in occurrence of *Cryptosporidium* oocysts among sample station at a 5% level of significance and statistical graphics on results from sewage and vended sachet water.

RESULT

A total of 59 samples, consisting of vented sachet water and sewage, were gathered from various locations in Maiduguri metropolis to detect *Cryptosporidium spp.* Among these, 51 (86.4%) were vented sachet water samples, while 8 (13.6%) were sewage samples, as illustrated in Table 1.

Table 2 presents the occurrence of *Cryptosporidium species* in vented sachet water samples, indicating a prevalence of 0 (0.0%). These samples were collected from multiple companies located in Maiduguri Metropolis, including AGK, Akko, Al-

Hindawiyya, Al-Maul Azbun, Al-Mustaf, Al-Uyun, A M G, Auwal, Bago, Bam, B G S, Biang Snow, Daloori, Demowa, Fachij, Fadimia, Fatimah, Feel Free, Habib, Hafees, Hamali, Hammadiyya, Haske, Hilu, Ibro, J & S, Jatau, Khalif B, Kwambaal, Linnass, Madube, Mahmud, Maisandari, Malakas, Nass, Nduruwa, Nurain, Patym, Qatar, Sadu, Safawa, Sha'aban, Tartifkata, Talbari, Tamam, Wajari, Yadoma, Zaimah, Zain, Zaituna, and Zayruss.

In Table 3, the prevalence of *Cryptosporidium species* in sewage samples collected from different areas in Maiduguri is depicted. These areas include Abbaganaram, Blind Workshop Bridge, Budum roundabout, Bulabulin gidanzana, Bulumkutu bayan makabarta, Gamboru Market Bridge, Kasuwan shanu Abbatoir, and Maduganari bayan tudu, with a prevalence rate of 3 (37.5%). The results indicate that 1 (12.5%) was identified from Blind Workshop Bridge, 1 (12.5%) from Bulabulin gidan zana, and 1 (12.5%) from Gamboru Market Bridge. However, no statistical significance was observed at ($p < 0.05$).

Table 4 outlines the prevalence of *Cryptosporidium spp* in both sachet water and sewage samples collected in Maiduguri metropolis.

Table1: Frequency Distribution of the Table Water and Sewage Samples Collected in Maiduguri

Sample	Frequency	Percentage (%)
V.S. Water	51	86.4
Sewage	8	13.6
Total	59	100

V.S. Water= vented sachet water

Table 2: Occurrence of *Cryptosporidium species* in Vended sachet Water

Location/Company	No. of Tested (%)	No. of Positive (%)
AGK, Akko, Al-Hindawiyya, Al-Maul Azbun, Al-Mustaf, Al-Uyun, A M G, Auwal	8 (0.00)	0.00
Bago, Bam, B G S, Biang Snow	4(0.00)	0.00
Daloori, Demowa	2(0.00)	0.00
Fachij, Fadimia, Fatimah, Feel free,	4(0.00)	0.00
Habib, Hafees, Hamali, Hammadiyya, Haske, Hilu	6(0.00)	0.00
Ibro,	1(0.00)	0.00
J & S, Jatau,	2(0.00)	0.00
Khalif B, Kwambaal	2(0.00)	0.00
Linnass,	1(0.00)	0.00
Madube, Mahmud, Maisandari, Malakas,	4(0.00)	0.00
Nass, Nduruwa, Nurain,	3(0.00)	0.00
Patym,	1(0.00)	0.00
Qatar,	1(0.00)	0.00
Sadu, Safawa, Sha'aban,	3(0.00)	0.00
Tartifkata, Talbari, Tamam,	3(0.00)	0.00
Wajari,	1(0.00)	0.00
Yadoma,	1(0.00)	0.00
Zaimah, Zain, Zaituna and Zayruss,	4(0.00)	0.00
Total	51(0.00)	0.00

Table 3: Occurrence of *Cryptosporidium* Species in Sewage Samples

Sewage Location	No. of Tested (%)	No. of Positive (%)	X ²	p-value
Abbaganaram	1(12.5)	0(0.0)	8.000	0.333
Blind Workshop Bridge	1(12.5)	1(12.5)		
Budum Round About	1(12.5)	0(0.0)		
BulabulinGidanZana	1(12.5)	1(12.5)		
Bulumkutu Bayan Makabarta	1(12.5)	0(0.0)		
KasuwanShanuAbbatoir	1(12.5)	0(0.0)		
Maduganari Bayan Tudu	1(12.5)	0(0.0)		
Total	8(100)	3(37.5)		

P<0.05

Table 4: Prevalence of *Cryptosporidium* species in sachet Water and Sewage Samples

Sample	No. of Positive (%)	No. of Negative (%)	X ²	p-value
Vended Sachet Water	0(0.0)	51(100)	1.054	0.304
Sewage	3(37.5)	5(62.5)		
Total	3(5.08)	56(94.91)		

DISCUSSION

Occurrence of *Cryptosporidium* spp. in sewage and sachet water was conducted in Maiduguri metropolis. 51 sachet water and 8 sewage samples were obtained from various locations within Maiduguri metropolis. Of the 59 samples taken, 51 (86.4%) are sachet water samples obtained from different locations in the study area, and 8 (13.6%) are sewage samples obtained from various locations within the Maiduguri metropolis. No sample of vended sachet water were found to be positive for the parasite this could be due to proper hygiene practice by the manufacturers while 3(37.5%) were found to be positive for *Cryptosporidium* spp in sewage sample. This was disagreed with the study conducted by Daraei *et al.*, 2021 in identifying the key contributing factors to *Cryptosporidium* spp in treated and untreated water which was 25.7% treated and 40.1% untreated, respectively. Also, the overall prevalence of *Cryptosporidium* spp. among all water types was defined as 36 (95% CI: 31.4-40.7). The

rank order of prevalence of *Cryptosporidium* spp. based on water type was wastewater (46.9%) > surface water (45.3%) > raw water (31.6%) > drinking water (25.5%) > reservoirs water (24.5%) > groundwater (18.8%) > swimming pool water (7.5%) > marine water (0.20 survival can help provide solutions at both local and global scales (Amorós *et al.*, 2016).

Method used in this study for discrimination of *Cryptosporidium* species was primarily applied to environmental samples in raw sewage samples and treated sewage Receptors Rivers in the United States (Xiao *et al.*, 2001). Also in a similar study, the results obtained are mainly about the species found in raw sewage: *C. muris*, *C. parvum* and *C. hominis*. PCR-RFLP has been applied in raw sewage samples in China, they have also reported the presence of *C. baileyi* and *C. suis*, and in 15.87% of the positive samples there were multiple species (Feng *et al.*, 2009).

The ability of the PCR-RFLP to discriminate species mixtures of *Cryptosporidium* in the

same sample has been demonstrated, nevertheless, it may be difficult in some samples as many species of the parasite may be present resulting in extremely mixed patterns (Ruecker *et al.*, 2005).

Identification of *Cryptosporidium* in sewage by molecular detection in Brazil 2019 was 76% which is higher than the present study that uses microscopy.

Results obtained in another study shows that *Cryptosporidium* oocysts and *Giardia* cysts were present in 26 of the 30 samples (86.6%) of raw sludge samples. In treated sludge samples, oocysts have been observed in all analysed (25 samples) with different stabilization treatment (83.3%). This study provides evidence that oocysts are present in sewage sludge-end products from wastewater treatment processes with the negative consequences for public health (Amorós *et al.*, 2016). The microbial diversity of wastewater used for irrigation and fertilization was assessed by Ben Ayed *et al.*, 2021 using specific polymerase chain reaction (PCR) assays to detect and genotype several pathogenic protists including *Cryptosporidium* spp., *Giardia duodenalis*, *Cyclospora* spp., *Eimeria* spp. and *Enterocytozoon bienersi*. A total of 220 wastewater samples (110 raw, 110 treated) and 12 sludge samples were collected from 2005 to 2008 from 18 treatment plants located throughout Tunisia. Except for *Cyclospora*, which was detected only once, *E. bienersi* (61%), *G. duodenalis* (28%), *Cryptosporidium* spp. (27%) and *Eimeria* spp. (45%) were frequently observed in wastewater and sludge. In recent time, no studies were

conducted on *Cryptosporidium* species in the study area.

CONCLUSION

A total of 59 vented sachet water and 8 sewage samples were collected from diverse locations in the Maiduguri metropolis. Out of the 59 samples, 51 (86.4%) were sourced from different table water producers in the study region, while the remaining 8 (13.6%) were sewage samples collected from various sites within the Maiduguri metropolis. *Cryptosporidium* spp. were not detected in the sachet water samples (0.0%), but a prevalence rate of 37.5% was recorded in sewage samples, specifically from Blind Workshop Bridge, Bulabulin Gidan zana, and Gamboru Market Bridge. It is noteworthy that proper hygiene practices were observed among the sachet water companies in Maiduguri metropolis, as no oocysts were identified in their products.

RECOMMENDATION

Based on the findings from this study, the following recommendations were made:

- I. It is shown that vended sachet water manufacturers are could be practicing good hygiene during the process.
- II. Reverse osmosis (RO) is the latest water filtration technique that is suggested to be used by sachet water manufacturers because it pull out clean water out of polluted or salt water by pushing it through a membrane under pressure despite there was no oocyst of the parasite detected from water.
- III. Sewage needs to be adequately treated before being recycled for use in other to prevent pollution.

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