Occurrence of Cryptosporidium species in surface water in South-eastern Nigeria: The public health implication

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Abstract

Using filtration, backwashing, concentration and modified Ziehl-Neelsen staining technique techniques water samples from major rivers serving for drinking and irrigation purposes were analyzed for Cryptosporidium oocysts in South-eastern Nigeria. Oocysts of Cryptosporidium species were found in all the sampling sites and there was significant variations` in the concentration of oocysts (F-ratio=3.367, P<0.05) with the highest mean oocyst concentration of 183.3 per litre of water while the least mean oocyst concentration of 120.6 per litre of water recorded. The highest mean monthly oocysts concentration of 226.5 per litre of water was observed in March while the least mean monthly oocysts concentration of 83.7 per litre of water was observed in the month of April. The differences in the mean monthly oocysts concentration per litre of water was statistically significant (F-ratio=3.23, P<0.05). As a public health measure, the development of public policies that limit contamination of surface waters is recommended.

INTRODUCTION

Cryptosporidiosis caused by the coccidian protozoan parasite Cryptosporidium species is a cause of morbidity and mortality in animals and humans. The parasite is responsible for an acute gastrointestinal and, less frequently respiratory infection in humans that is self-limiting in immunocompetent people but prolonged and potentially life-threatening for the immunocompromised population ([1]). Cryptosporidium is transmitted by oocysts, passed in the faeces and these oocysts are remarkably resistant to common disinfectants and can survive for several months under cool, moist conditions ([2]). Oocysts are present in many environmental waters because Cryptosporidium is not only a human pathogen but also a zoonotic pathogen infecting livestock, as well as feral animals, in many watersheds used as sources of drinking water ([3]). Surveys of surface water, groundwater, estuaries, and seawater have dispelled the assumption that Cryptosporidium oocysts are present infrequently and in geographically isolated locations and indicate that water is a major vehicle for transmission of cryptosporidiosis ([1,4]).

Water-borne transmission is facilitated by the small size of the oocysts (3.5-5.0µm), suboptimal processing of water treatment facilities and long lasting infectivity of the oocysts in the environment ([5]). Numerous studies have reported the contamination of surface water by Cryptosporidium. Sources of contamination of surface waters include sewage effluent overflows, waste-water discharges, abattoir waste, direct animal faecal deposition in waterways, indirect deposition via runoff from land grazed by livestock and/or wildlife, manure and effluent spreading, and storm water run ([6,7,8,9]). Although person-to-person contact and domestic animals are some of the possible sources of infection, exposure to and/or consumption of contaminated drinking water and the use of surface waters for recreational activities are among the most important routes of transmission of the parasite ([10,11]). Infected individuals may excrete many millions of oocysts each day during the acute stage ([12]). The implications of Cryptosporidiosis in persons with HIV infection have been reported. AIDS patients are known to excrete up to 10^{10} Cryptosporidium oocysts per day and in AIDS- related diseases, Cryptosporidium ranks only second to tuberculosis as a terminal disease in HIV infection ([13,14]).

There is paucity of information on the occurrence of Cryptosporidium in surface waters in most parts of Africa including Nigeria. Studies on these parasites in the environment are still limited due to absence of epidemiological evidence and difficulty of adequate methodologies of sampling and analysis ([4,5]). Because detection of Cryptosporidium oocysts in raw water sources is considered an important component in the management,

prevention, and control of Cryptosporidium in drinking water supplies ([13]), the purpose of this investigation therefore was to examine surface waters for the occurrence and distribution of Cryptosporidium species. The public health implications of findings and the need for improved water quality regulations are discussed as they affect Nigeria and other developing countries of similar setting, particularly in Africa.

METHODS

STUDY AREA

The study was conducted from December 2004 through May 2005 in Abakaliki one of the major cities in the south-eastern Nigeria and capital of Ebonyi State. The area lies approximately between longitude 8°6'6" E and latitude 6°22'26" N and is located on the lower belt of the Niger. The climate is tropical and the vegetation characteristic is predominantly the semi-tropical rain forest with an average annual rainfall of about 1600mm and average atmospheric temperature of 30°C. There are two distinct seasons; the wet and the dry seasons. The former occurs between April and October, while the latter takes place from November to March.

The area is traversed by a number of rivers which include Iyiudele River, Iyiokwu River, Ebonyi River and Okpuru River and form a confluence at the Southern part of the city. These rivers constitute the major sources of water supply especially to the suburbs and rural communities bordering the city. Water from these streams and rivers serve for drinking, washing, bathing and irrigation purposes for the medium and small-scale farming which is a major occupation of the people inhabiting the suburbs of Abakaliki where many varieties of the vegetables and fruits sold in the city are grown. The rivers and other water courses drain the city collecting both human and animal wastes from homes, hospitals, markets and industries and are often polluted with organic substances with higher concentrations during the dry season.

SAMPLE COLLECTION

Water samples were collected from four different locations in four different rivers in the study area (Iyiudele River, Ebonyi River, Iyiokwu River and Okpuru River). At each sampling site, 50 litres of water sample was collected per week randomly from the respective water bodies in plastic containers and transported immediately to the Medical Microbiology/Parasitology Laboratory of Ebonyi State University, Abakaliki, for analysis. The sampling was carried out for the period of five months giving a total of 1,000 litres to water samples per sampling site and a total of 4,000 litres in all.

SAMPLE ANALYSIS

Analysis of samples was done using the techniques described in previous studies with slight modifications ([15,16,17]).

Filtration: The water samples were filtered as soon as they were collected. The sample was passed through a 60mm and 90mm mesh to remove big debris. It was then filtered using a white khaki cloth (cotton) as filters (mesh size 250 – 300µm) with the aid of a vacuum pump and a Buckner filter flask. The cloth was cut into 8 cm^3 pieces, and to enhance an effective trapping of particles and the organisms during the filtration process; five of each piece was placed together in the funnel thus forming five layers. When the pores are blocked during the filtration process as indicated by the slow flow rate of filtrate into the Buckner filter flask, the topmost layer of the cloth was removed and another added at the bottom thus, maintaining the five layers at any point in time as the filtration continued. All the filters, which had trapped minute particles, were collected into marked polythene bags and stored in the refrigerator to keep them moist.

Backwashing: The filters were eluted by several backwashing with 200 ml of distilled water in which 10% wash solution concentration had been incorporated so as to enhance the recovery of oocysts. The wash solution was prepared using distilled water and Tween 20 (Sigma Chemical Co., St. Louis, Mo.).

Concentration: The sucrose gradient sedimentation technique was used for the concentration of oocysts. After the backwashing, 9 ml of the water sample was placed in a conical centrifuge tube and 2 ml of Sheather's sugar solution was added. This was stirred vigorously and centrifuged at 3000 rpm for 10 minutes. The supernatant was carefully removed until about 2 ml is left with the sediment in the centrifuge tube. The sediment was transferred to the microscope slides and smears made.

Microscopic Examination: Demonstration of Cryptosporidium oocysts was by microscopic examination of smears made after the concentration of water samples, fixed with 70% methanol and stained using the modified Ziehl-Neelsen staining technique ([18]). The air-dried smears

were fixed in 70% methanol for 3 minutes. This was then stained with cold Carbol-fuchsin for 10 minutes after which the stain was washed off with clean tap water. The smear was then decolorized using 3% hydrochloric acid (HCl) in 95% ethanol until no more colour floods from the smear. The decolorized was rinsed off with tap water and the smear was counterstained with 0.25% weight per volume malachite Green for 30 seconds. The stain was again washed off with water and the smear was allowed to dry. The x40 objective was used to examine the smear for identification of oocysts.

Visual oocyst count: The number of oocysts was determined by scanning through each slide randomly. This was done by moving three different parts of he slide each at a time across the x10 objective and looking out for the pinkish stained oocyst. Oocyste encountered were confirmed using the x 40 objective, by their oval or round shape and pink-red coloration. These were counted and their numbers recorded.

STATISTICAL ANALYSIS

Differences in means were evaluated by the one-way ANOVA described previously ([19]). The level of statistical significance was set at 0.05.

RESULTS

Oocysts of Cryptosporidium species were found in all the sampling sites. The water samples collected from Ebonyi River were found to have the highest occurrence of Cryptosporidium species with mean oocyst concentration of 183.28 per litre of water while samples from Okpuru River had the lowest mean oocyst concentration of 120.58 per litre of water (Table 1). There was a statistically significant difference in the association between the mean oocyst concentration and sampling site (F-ratio=3.367, P<0.05)

In relation to the period of sample collection, variations in oocysts concentration were observed. Samples collected in the month of March had the highest mean monthly oocysts concentration of 226.47 per litre of water while the least mean monthly oocysts concentration of 83.69 per litre of water was observed in the month of April. The mean monthly oocysts concentrations of 124.14, 157.06, 178.8 and 156.31 were observed in December, January, February and May respectively (Table 1). The differences in the mean monthly oocysts concentration per litre of water was statistically significant (F-ratio=3.23, P<0.05).

Figure 1

Table 1: Mean concentration of oocysts (per liter of water) in the surface water in Abakaliki, Nigeria

	Study Period													
Rivers	December		January		February		March		April		May		Summary	
studied	ES	LS	ES	LS	ES	LS	ES	LS	ES	LS	ES	LS	Total	MPP
Ebonyi	156.3	163.7	178.4	178.6	230.6	230.8	252.3	253.7	104.0	104.0	168.4	178.6	2199.4	183.3
Iyiudele	130.4	130.6	159.2	160.8	210.7	210.7	238.3	240.2	80.6	73.4	159.7	160.3	1955.0	162.9
Iyiokwu	132.6	138.4	162.6	162.4	210.8	210.9	241.2	244.6	96.4	90.6	152.6	178.4	2021.5	168.5
Okpuru	96.6	44.5	124.6	129.9	168.5	168.5	168.8	172.6	67.2	53.3	124.6	127.9	1447.0	120.6
Total	515.9	477.2	624.8	631.7	820.6	820.9	900.7	911.1	348.2	321.3	605.3	645.2	7622.9	
MPS	129.0	119.3	156.2	157.9	152.5	205.2	225.2	227.8	161.3	80.3	151.3	87.1		
MPM	124.14		157.06		178.83		226.47		83.69		156.31			

Key: ES= early sampling; LS=late sampling; MPP=mean per sampling period; MPS=mean per site; MPM=mean per month.

DISCUSSION

The very high concentration of Cryptosporidium oocysts in water samples from all the four sample sites confirmed the presence of Cryptosporidium species in surface waters in Abakaliki, South-eastern Nigeria. This finding is in conformity with previous reports which noted that the oocysts of Cryptosporidium species were continually present in virtually all surface waters ([20]). In this present study however, the oocysts concentration were high just before the onset of the rainy season. The concentration gradually increased from December (the onset of dry season) and reaches its peak by March. There was an observed decrease in concentration of Oocysts after the first rainfall in April and a slight increase as the rain stabilizes in May. Variations in oocysts concentrations have been shown to be associated with and were highest when they were influenced by post rainfall run-off and showing decrease through the summer months ([20,21]). Thus this increase in the concentration of Cryptosporidium oocysts in water could be due to rainfall besides a statistically significant difference was observed in the trend.

It is already well established that millions of oocysts are usually excreted by an individual (approximately 10^6/ml of stool each day) during an acute stage of cryptosporidial infection and patients with AIDS are known to excrete approximately 10^{10} oocysts per day ([12]). Hence in the study area during the dry season, there is usually little water for flushing the human and animal faeces carrying the oocysts from the surfaces/environment into streams or larger water bodies. Therefore small volumes of water may wash this massive number into the rivers during the dry season resulting to the high concentration of oocysts at such periods ([17]). In addition, the ocysts are carried slowly and distributed

along the water course and at the peak of the rains, they are dispersed and washed off as rivers flows downstream ([5,21]). This may explain the occurrence of high concentration of oocysts in this investigation during the month of March (an average of 226.47 oocysts per litre of water).

The highest concentration of oocysts was recorded in Ebonyi River, with a mean of 183.28 oocysts per litre of water. This could be because Ebonyi river is the downstream of Iyiudele River which drains the major parts of the study area and carrying most of the human and animal wastes especially wastes from the major markets (Kpirikpiri market, Eke-Aba market, Abakpa market) and from homes of people of low social and economic status living predominantly in the stems along this river course and have no proper waste disposal facilities. It is well established that there is usually high concentration of Cryptosporidium oocysts in the surface waters located in areas where there is inadequate waste disposal system ([10,12]). The Okpuru River recorded the least mean concentration of Cryptosporidium oocysts (120.58 oocyst per litre of water). This could be attributed to the sparse nature of the human settlements around the River. In addition the river course is devoid of outlet channels and this makes the water unfavourable for settling of Cryptosporidium oocysts as noted in previous studies ([7,20]).

It is important however to state that although the modified oocyst isolation technique used in this study was reasonably sensitive, the possibility of underestimation may not be ruled out. More accurate and sophisticated techniques for the isolation of Cryptosporidium oocysts from surface waters including Method 1623 and Cell culture PCR have been described ([22]). However these techniques are expensive and obviously beyond the reach of researchers in most parts of Africa. This study therefore recognized the use of modified Ziehl-Neelsen staining technique as effective and sensitive for the detection of Cryptosporidium oocysts in water samples. This is in agreement with the recommendation of a previous study conducted in Egypt ([23]).

In conclusion, the presence of high concentrations of Cryptosporidium oocysts observed in surface waters in this study suggests that there is a high potential of Cryptosporidial infection to both humans and animals in the study area. This calls for urgent public health interventional efforts because studies have shown that the infective dose for cryptosporidium oocysts is small. In a dose-response study, it was demonstrated that as few as 30 oocysts initiated infection in immunocompetent individuals ([24]). The epidemic potentials of Cryptosporidial diarrheoa and the implications of cryptosporidiosis in HIV/AIDS and immuno suppressed individuals have been documented ([25,26]). The results of this study suggests that vegetables and fruits harvested from the farms especially during the dry season may be contaminated with Cryptosporidium oocysts, since water at the downstream is often used for irrigation of these farms. As a result consumers of farm produce especially raw fruits and vegetables commonly sold in the markets must to take necessary precautions before consuming such items to avoid cryptosporidial infection. Public health officials should consider a communication program to physicians treating the immunocompromised, nursing homes, develop a plan to evaluate cases of cryptosporidiosis in the community, and contribute to the development of public policies that limit contamination of source waters, improve water treatment, and protect public health.

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