International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 3, p. 28-39, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Experimentation of immunofluorescence technology in the quantification of *Cryptosporidium* spp. and *Giardia* spp. (oo) cysts in surface water in Yaounde, Cameroon

Ajeagah Gideon A*, Foto Samuel, Njine Thomas

Laboratory of Hydrobiology and environment, Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde 1, P.O Box 812, Cameroun

Received: 10 February 2012
Revised: 24 February 2012
Accepted: 26 February 2012

Key words: *Cryptosporidium* spp. oocysts, *Giardia* spp. cysts, USEPA Method 1623, surface water, Yaoundé.

Abstract

With the advent of the Acquired Immune Deficiency Syndrome (AIDS), the human host has become susceptible to most opportunistic pathogens. Some of these organisms are the emerging protozoan entero-parasites; *Cryptosporidium* spp and *Giardia* spp. The aim of this study was to isolate and identify the oocysts of *Cryptosporidium* spp and cysts of *Giardia* spp in the surface water of Yaoundé, Cameroon, using the standard(United States Environment and Protection Agency) USEPA Method 1623, which is based on ultrafiltration, immuno-magnetic seperation and fluorescence antibody. The analysis lead to the determination of some ecological factors of the aquatic ecosystem, which are turbidity, suspended solids , ammonia, Biochemical Oxygen Demand and the correlation between the biodynamics of the (oo) cysts in the medium characterised. These particles could play an important role in the transportation of the (oo) cysts in the aquatic medium by an adsorption mechanism. Our results obtained for the surface water suggested an increase in the pathogen densities from upstream to downstream. The minimum-maximum values recorded for the for *Giardia* cysts along the Mfoundi , were 7-15 cysts/L , 12-38 cysts /L and 31-98 cysts/L respectively. The minimum –maximum values recorded for the *Cryptosporidium* oocysts from upstream to downstream of the Mfoundi, were 4-6oocysts/ L, 18-54 oocysts / L and 26- 64 oocysts/ L respectively.

*Corresponding Author: Ajeagah Gideon A ✉ ajeagahg@yahoo.com

Introduction

Cryptosporidim spp and *Giardia* spp have been recognised as the major agents of foodborne and waterborne diseases outbreaks associated with faecal contamination (Doron, 2000).They are resistant to chlorine disinfection at high infectivity, they are zoonotic and impair the development and socio economic potentialities of the host , they have been included in the World Health Organisation(WHO) list of neglected diseases initiative (Savioli *et al.,* 2006).

The species of the genera *Cryptosporidium* and *Giardia* are enteroprotozoan which cause Cryptosporidiosis and Giardiasis respectively. They can be fatal for people whose immune systems are deficient. In immunocompromised host ,the infection is chronic and the number of parasites soar, resulting in the invasion of the secondary sites such as the duodenum, large intestines, stomachs, biliary and pancreatic ducts and the respiratory tracts. They have been identified in multiple parasitic outbreaks in the United States of America according to Craun *et al.,* 2010 and in Korea as presented by Mok-Young *et al.,* 2011.

The identification of the these oocysts in water samples is problematic due to their small size and varying morphology. The lack of morphological characters to discriminate *Giardia* and *Cryptosporidium* variants has limited the understanding of the taxonomy ,epidemiology and public health significance of these clinically important genera .These organisms lack host specificity and sometimes the concentration of oocysts in surface water and waste water in developing countries is mostly associated to human and animal waste as stipulated by Savioli *et al.,* 2006.According to Keush *et al.,* 1995, oocysts can maintain their infection power for long periods in water. *Giardia* is a flagellated protozoa, it has three pairs of flagella in the trophozoite stage ,giving it a high mobility in the intestinal tract, while the sporozoites of *Cryptosporidium* enables a high penetration power in the host cells, resulting in intestinal disorder.

The apical organelles of *Cryptosporidium* which are micronemes, rhoptries and dense granules, contain a complex mixture of proteins that are either secreted into the apical end of the zoite or into the digestive apparatus whose function include adhesion, specific attachment to the host cell, gliding and locomotion. The transmission between humans and animals have been supported by cross infection studies, however a closer examination of many studies have revealed limitations in the methodologies that are applicable in accordance with the findings of Dai and Boll, 2003; Harter *et al.,* 2008. Most recent molecular genetic studies have demonstrated considerable diversity among isolates of the same species of *Giardia* and *Cryptosporidium*, suggesting that these species are infact species complexes and that some of these novel species may be host specific.

There is a very high risk of morbidity and mortality from these gastrointestinal diseases because of the high prevalence of the human immune virus, cancer chemotherapy and organ transplants. Cross studies have demonstrated the possibilities of transmission between host species. It has also been proposed that *Cryptospordium* spp be considered a monotypic genus, these enteropathogens are sometimes considered as a primary pathogen in most diseases that are associated with the release of liquid stool (Giovanni *et al.,* 2006), The knowledge of the biology of emerging waterborne parasites and their transmission routes, pathogenicity, dose response ,zoonotic transmission is needed in combination with a worldwide information exchange system evaluating the significance of the different sources for the occurrence of oocysts at a specific surface water site is determined by a combination of factors such as the contamination level of these sources, the parasitic load, the transport potentialities of the oocysts ,the physico-chemical

variables of the medium and the possibilities of the oocysts in the environment(Brooks *et al.,* 2006).

Many studies carried out around the world have indicated the presence of *Giardia* spp and *Cryptosporidium* spp in waste and water surface water and underground water (Arnone and Walling, 2006). Very few studies have been carried out in developing countries with the application of a more recommended methodology. The aim of this study is to isolate and identify the oocysts of *Cryptosporidium* spp and *Giardia* spp oocysts in an urban mainstream, based on the standard USEPA Method 1623 which is based on immunomagnetic separation and epifluoresecence antibody, then relate the biodynamics to the ecological factors of the medium.

Materials and methods

Geographical location and description of the Yaoundé Watershed

The city of Yaoundé is located on the western border of the South Cameroon Plateau at an altitude of 3°52'N and a longitude of 11°32'E. This plateau has an average altitude of 750m (Bachelier, 1959). The climate in Yaoundé according to Suchel (1972) is of the equatorial type, it is hot and humid but attenuated by the altitude. This climate is characterised by moderate precipitations (annual pluviometry mean: 1576 mm) and temperature varies slowly with time from 22.4 to 27.4°C. The highest daily thermal amplitude is 10.4°C recorded in February, while the lowest value is 7.2 °C and is recorded in July. December, January, February, July and August are the sub-arid months, while April, May, September and October are the months with the highest rainfall. Four seasons can be distinguished in Yaoundé; a long dry season (from December to March), a short rainy season(from April to June), a short dry season(July and August) and a long rainy season(September to November). Some of the streams of Yaoundé belong to the Mfoundi River Basin, which is made up of many tributaries which are mostly permanent. These streams flow into the Mfoundi mainstream which bears the name of the river basin.

Surface water sampling sites

The Mfoundi stream is the main watercourse that flows across the city of Yaoundé. The other streams such as Akeu, Biyeme, Ebogo, Ekozoa, Ebogo, Ewoue, Mingoa, Nkie, Ntem, Odza, Olezoa, Tongolo and Osegui are the major tributaries of this stream. The Mfoundi stream takes its rise from Amang Oliga (Parcours Vita), and then crosses Mvog-Yidi, Ekoudou, Mballa 1, Nkol-Eton, Mfoundassi, Etoa–Meki, Elig-Essono, Mvog-Mbi, Dakar and Nsam quarter, before joining the Nyong river at Mbalmayo.The water of stream is used for urban agriculture, fishing, domestic exploitation and the production of potable water. They are more than five hundred thousand people living along the banks of the river.They are more houses constructed upstream and some industrial effluent that open downstream of the Mfoundi. Three sampling points were selected from upstream to downstream.

Mfoundi 1(M1): This point is located at Ekoudou in the Yaoundé I sub- division, just where the river crosses the Mount Febe road. This point was chosen because of the accessibility to the site and the low human activity around the point. There is no tributory upstream, so the values of the (oo) cysts densities will give an idea of their presence in a lowly polluted aquatic ecosystem.

Mfoundi 2(M2): This site is located at some 500 m after the Central Post Office at the Yaoundé V sub-division. It gives an idea on the quantity of the resistant form of the parasite that is added by other watercourses such as Tongolo, Ntem, Ekozoa and Abieurgue West. There is a higher level of anthropogenic activity, and also the possibility of contamination of the ecosystem by peril fecal matter. The release of organic matter and wastewater by the urban households increases the contamination of the river by these pathogenic micro-organisms.

Mfoundi 3(M3): This site is located at Dakar, just below the Brasseries du Cameroun in the Yaoundé IV sub-division. Here, one can observe an accumulation of waste matter from a majority of the streams in the urban river basin. The slaughter house of the Yaoundé Municipality is located just before this sampling point. It is possible that the (oo) cysts sampled at this point come from a zoonotic and anthroponotic source..

Physico chemical analysis

Water samples of 20 liters each was analysed during each sampling process of the isolation and identification by the USEPA method 1623.Twenty four samples were collected, twelve for biological analysis and twelve for physico-chemical assessment. The principles and apparatus applied in the analysis of environmental variables is that elaborated in the standard methods for the assessment of water and wastewater, APHA,, The Temperature was assessed by thermometry, conductivity and Total Dissolved Solids by conductimeter ,pH by a pH meter, Oxygen, Carbondioxide, alkalinity,chloride by volumetry. Calcium, total hardness and Magnesium hardness by complexometry then volumetry, BOD5 by respirometry and Oxydability by a reduction of Potasium permanganate using the organic matter in the sample. The Mortimer scale was employed in the characterisation of the Oxygen percentage in the medium, while phosphates were quantified by spectrophotometry.

Collection and experimentation of immunomagnetism and epiflorescence microscopy

Each water specimen was collected in a sterile 20 liter polyethylene container from 7a.m to 11a.m .Those in the Municipal lake were collected with the aid of a Van Dorn bottle. All the specimens were transported at ambient temperature to the laboratory of General Biology of the University of Yaoundé 1, where they were then subjected to the isolation and detection of *Giardia* spp. cysts and *Cryptosporidium* oocysts, in the 45 minutes that followed the field sampling. The isolation of (oo) cysts by USEPA Method 1623 took place from June 2006 to August 2006.The experimental setup for the isolation and identification of (oo) cystsis presented in Fig. 1.

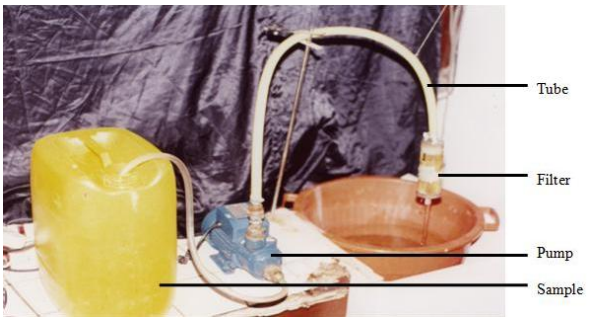

Fig. 1. Experimental disposition of the ultra-filtration mechanism with an Envirochek filter.

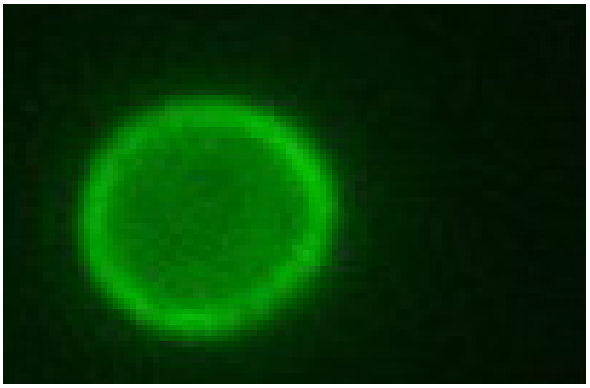

Fig. 2a. FITC- conjugated image of *Cryptosporidium* sp. oocysts (Fluorescence structure).

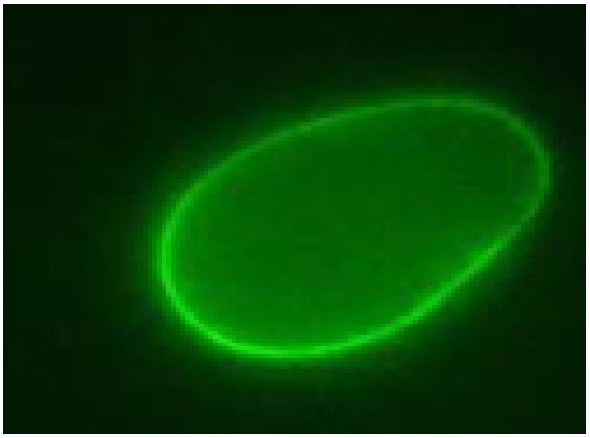

Fig. 2b. FITC– conjugated image of Giardia sp cysts (Flourescence structure).

Filtration of the samples: The Envirochek sampling capsule was applied in the concentration of *Giardia*

cysts and *Cryptosporidium* oocysts. This capsule which meets standards as listed in U.S. EPA Method 1623, has a capacity of 1300 cm^3, with dimensions of 21cm in length and 6cm in width. It is mounted on a polyethersulfone membrane with a polypropylene filter support material. It can sustain a differential pressure of 2.1 bar and it is provided non sterile, the filter is non reusable.The water sample is pumped through the inlet by an electrically powered pump as represented in figure 1 below, it passes from the outside (visible) surface through the membrane to the inner core and is expelled through the outlet where suspended solids greater than 1um in size is deposited on the outer surface of the membrane. The filter is particularly designed to allow easy recovery of the deposited matter. It includes vinyl caps that fit over the hose-barbed inlet and outlet to form a water tight seal for sample conservation and the elution process. A standard male luer lock (Bleed valve) fitting is used to purge air out from the capsule as it fills with water. Before the Envirochek filter is connected to the system, the pump is turned on, so as to clean the whole system of any residual debris that may affect the system. Ten liters of sample are then allowed to be filtered. The residual air in the capsule is expelled by turning the bleed valve in the counter clockwise direction. When the filter housing is full of water the bleed valve is closed. After the process of filtration, the capsules are sealed by the venyl caps, and are ready for the recovery of the resistant forms of the pathogens.

Elution of the (oo) cysts: An elution buffer is applied in the recovery of the (oo) cysts. This buffer consists of Laureth-12, Tris at a pH 7.4 which is prepared by using hydrochloric acid and deionised water. EDTA at pH 8.0 is also prepared using sodium dihydrate. Antifoam A is also applicable in the preparation of the final buffer. The buffer is added through the inlet end of the capsule and the liquid level is allowed to stabilise. Enough buffers are added in order to recover the pleated white filter module. The green venyl end cap to the inlet end of the capsule is replaced. The capsule is hand agitated for five minutes, then the inlet end cap is carefully removed and the contents of the capsule are distributed in 15 ml centrifuge tubes ready for centrifugation.

Centrifugation: The sample is spin at 1500 X G for 15 minutes, using the Medfirger centrifuge. The centrifuge is allowed to coast to a stop. A Pasteur pipette is used to carefully remove the supernatant to just above the pellet. The pellets in all the tubes are collected in a single tube and the volume in some cases is adjusted with the aid of reagent water to make a total pellet volume of 10 ml which is ready for the immuno-magnetic separation of the *Giardia* cysts and the *Cryptosporidium* spp. oocysts.

(Oo) cysts separation: The rapid and selective separation of Giardia cysts and Cryptosporidium oocysts from water samples concentrates was carried out with the aid of Dynabeads GC-Combo. These are uniform, mono-disperse super-paramagnetic and microscopic beads with purified antibodies against Cryptosporidium oocysts and Giardia cysts covalently bound to the surface. They are supplied as a suspension in phosphate buffered saline (PBS), pH 7.4 with 0.1 percent Bovine Serum Albumin (BSA) and 0.02 percent Sodium Azide (NaN3).These beads are conducted in a buffer system which has been specifically designed for the efficient separation of both Giardia cysts and Cryptosporidium oocysts from a wide range of water types. These are the 10X SL buffer A, which is a clear colourless solution and the 10X SL Buffer B which is the Magenta solution. They are both stored between 2-8°C and are equilibrated to room temperature (15-22°C) before usage. All Giardia cysts and Cryptosporidium oocysts which react with the antibodies bound to the beads will be isolated using Dynabeads GC- Combo. This specificity tests limits the degree of non-specific binding with Dynabeads GC Combo due to the use of the SL buffer. This product will perform satisfactorily in treated or untreated water samples that are destined for potable water supply.

Principle of action: Dynabeads G-C combo are designed for rapid, selective separation of Giardia cysts and Cryptosporidium oocysts from water sample concentrates using IMS. This reagent replaces the flotation techniques currently used for separating the cysts and oocysts from other debris in water samples concentrates. The paramagnetic beads are incubated with the water sample concentrates along with the SL buffer. The Antibodies coated on the beads selectively bind cysts and/or oocysts within the water sample concentrate and form a complex. The Dynabeads–organism complexes are separated using a dynal magnetic particle concentrator (Dynal MPC) and subsequently the cysts and oocysts are dissociated from the beads. There are two types of Magnetic Particle Concentrators that are applicable in the process of immuno-separation. These are the Dynal MPC-1 and the Dynal MPC-S.

The Dynal MPC-1 is used to separate dynabeads from diverse liquid sample matrices. The beads are attracted to the magnet adjacent to the tube wall when the tube is inserted into the dynal MPC-1. This enables easy removal of the supernatant while the Dynabeads are left isolated in the tube. The Dynal MPC-1 is made from disinfectant proof polyacetate equiped with dynal biotech rare earth magnets (Neodymium-Iron – boron permanent magnets). It has been designed to hold one single tube with variable diameters that are up to 30mm and volumes that vary from 5 to 50ml.The remanence of the magnet (Br) is 12.200 tesla, with a coercive force of 11.400 Oersteds 410KA/m . It has a high intrinsic coercive force and functions as a Maximum Energy Product. These permanent magnetic properties ensure the satisfactory isolation of immuno-magnetic beads as presented in Fig. 1.

The dynal MPC-S is also used to separate Dynabeads from diverse liquid sample matrices. The beads will be attracted to the tube opposite wall when the tube is inserted into the Dynal MPC-S housing and the magnetic slide is inserted. This enables the easy removal of the supernatant .The Dynabeads are left isolated in the tube. It is made of desinfectant proof material for easy cleaning. The removable magnetic slide contains optimal rare – earth neodymium permanent magnets, while the blue plastic strings contain maximum structural memory characteristics. It is designed to hold 6 microcentrifuge tubes which have a working volume ranging from 10 µl to 2 ml. The slide is inserted with the protruding edge downwards and the table side facing the tubes. It is made of high performance rare earth magnetic alloy neodymium iron boron (NDFeB) , with a residual flux density (Br) of 12.200 Gauss (1.22 Tesla).The tilted position for the insertion of the slide is applicable in the isolation of *Giardia* spp. cysts and *Cryptosporidium* spp. oocysts depend on the avidity of the anti-bodies or ligands on the surface of the Dynabeads as well as factors concerning the biomolecules themselves and the matrix from where they have been isolated. In our study the volumes ranged from 0.01 to 0.5ml. The resultant suspension for screening is clean and a small volume of about 50 µl

Immunoflorescence antibody (IFA) detection of (oo) cysts: The slide is screened by an epiflorescence microscope of mark Leitz at the 100 objective. The *Giardia* cysts and *Cryptosporidium* oocysts emit an apple green florescence upon observation.The morphology of Giardia is oval, while that of *Cryptosporidium* is round. The *Giardia* cysts appear larger (7- 12µm) than *Cryptosporidium* (4-6um). The (oo) cysts are counted at the various sampling points. The morphology and the apple green fluorescence emitted by the oocysts are compared with those of the positive control samples provided by the Cell lab company, along with the antibodies. The colour produced by FITC is superimposed on the wall of the cyst and oocysts. The fluorescence of the rim is crisp and sharp; it has a consistent width around the entire organism. The interior fluorescence is dimmer than that of the rim. There is no scattering of fluorescence

when focussing. One fluorescing rim is observed around the cysts and oocysts.

Statistical analysis

The pearson correlation coefficient was calculated for *Cryptosporidium* spp oocysts population dynamics and Giardia spp cysts dynamics identified in the surface water by immunoflourescence separation. It was also employed in the assessment of the relationship between physical and chemical variables at the various sampling points of the Mfoundi River Basin. The SPSS package was considered in the analysis, at a 5% confidence interval.

Results and discussion

Physico-chemical parameters

The ecodynamical variables are presented in Table 1a and b. Conductivity and Total Dissolved Solids (TDS), increases from upstream to downstream, with values ranging from 185,22±19.17 to 267,28 ±37,03 uS/cm ; 92.28±98,72 to 133.21 ± 43.66 mg/L.pH was slightly acidic in the aquatic ecosystem, while the temperature ranged from 22-25 oC along the streamcourse.Suspended solids were highest at Mfoundi 2, with values ranging from 284.72 to 308.88 mg/L. as presented in Table 1.

There was a very low oxygenation of the Mfoundi mainstream , giving a`saturation percentage that is below 30 percent.Carbondioxide, Ca, Ca + Mg, Cl-, both increased from upstream to downstream as presented on Table 2.The indicators of organic pollution attend maximum values of 22.02± 13,88 mg/L for oxydaility and 97.5± 4.82 mg/L for (Biochemical Oxygen Demand) BOD. The phosphate content which is an indicator of the eutrophication of the waterways had a maximum values reaching 6.49 ± 1.59mg/L.

Morphometry and microscopic observation of pathogenic agents isolated

Cryptosporidium *spp*

The oocysts are round as illustrated in figure 2a. The diameter varied from 4 µm to 6µm with an average of 5 µm for 100 observations. The fluorescence of the crisp is sharp; it has a consistent width around the entire oocysts. The interior fluorescence is dimmer than that of the rim. There is no scattering of fluorescence when focussing. The *Cryptosporidium* oocysts recorded are Mfoundi 1 (4-12), Mfoundi 2 (18-54) and Mfoundi 3(26-66) (oo) cysts/L

Giardia *spp*

Giardia cysts have a characteristic oval structure as analysed in figure 2b. The dimensions of the width vary from 7 µm to 8 µm with an average value of 7.5 µm, while the dimensions of the length vary from 11 µm to 12 µm with an average value of 11.5 µm for 100 observations. The fluorescence of the membrane is crisp and sharp; it has a consistent width around the entire organism . The interior fluorescence is dimmer than that of the rim. The *Giardia* cysts recorded are Mfoundi 1 (6-16), Mfoundi 2 (12-75) and Mfoundi 3(31-99) cysts/L.

The analysis of the correlation coefficient reveal a positive and significant value for the oocysts/cysts densities which is 0,890,(oo) cysts/Physical parameters and the (oo) cysts chemical parameters(Table 2).The bio-dynamics of the resistant forms of Cryptosporidium spp and *Giardia* spp were assessed in the Mfoundi mainstream in the short dry the rainy season.The results obtained suggests the ubiquity of C*ryptosporidium* and *Giardia* oocysts in this watercourse.The population density of Giardia cysts increase from upstream to downstream .The lowest density of cysts was recorded upstream, in the month of June while the highest cysts density of filtered water was assessed downstream of the Mfoundi mainstream.

The values of *Cryptosporidium* spp analysed in the river basin follow a similar trend to that of *Giardia* spp

cysts,eventhough there is a lower numerical counts for the *Cryptosporidium* oocysts with respect for the *Giardia* cysts. The average values of the transmissive form of *Cryptosporidium*, isolated and identified in the Mfoundi mainstream increases from upstream to downstream of the Mfoundi mainstream, the lowest average value recorded was at the upstream, while the highest average value recorded of surface water sampled in the Mfoundi was downstream.

The values obtained reveal an isolation and identification of *Cryptosporidium* spp and *Giardia* spp in the Mfoundi River Basin in all the sample points(Fig. 3).There is a remarkable increase in the resistant forms of parasites as in the Mfoundi mainstream as the number of tributaries transporting the pathogenic micro-organismes increase along the river course(Figure 3), there is an increase of emission set points such as toilets and the slaughter house as indicated by the very high values recorded for the organic and inorganic indicators of contamination in the aquatic medium(Table 1 a and b). The calculated value of the Pearson correlation coefficient between the cysts and oocysts distribution gives a very high and significant relationship for a $p \geq 0.05$ as associated to the meteorological conditions by Wuhip *et al.*, 1994 (Table 2). An investigation of the Pearson correlation between the physical/physical and chemical/chemical ecodynamical variables present positive and highly significant values for the various variables investigated at the different biotopes of the Mfoundi rivers basin (Table 2)

Giardia cysts and *Cryptosporidium* oocysts were detected in all surface water samples which both include the filtration of a large volume of water through a cartridge filter; elution of cysts and oocysts from the filter matrix(Fig. 2). Centrifugation and identification by the genus specific FITC labelled monoclonal antibodies epidemic episodes are mainly due to the spread of new agents or newly identified infections such as *Cryptosporidium* sp and *Giardia* sp as identified in the samples analysed (Fig. 2 and 3).

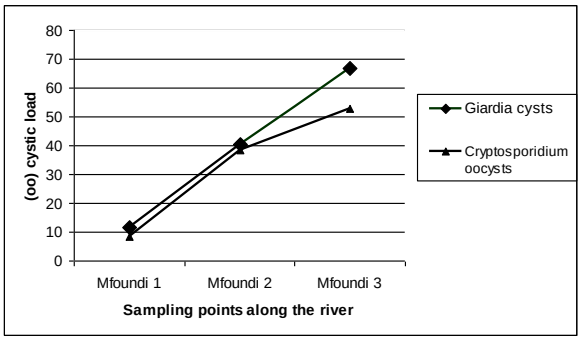

Fig. 3. Dynamics of transmission of (oo) cystic load in the Mfoundi mainstream

Surface water and drinking water plays an important role in the transmission of the diseases (Arnone and Walling,2006; Harter et al,2008, Kabore *et al.*, 2010). The river system is contaminated with faecal-oral transmitted parasites, strategies incorporating health education regarding personal hygiene ,appropriate disposal of toilets need to be embraced by this community in order to control the spread of these parasites(Brandinosio *et al.,* 2000; Craun *et al.,* 2010, Mok-Young, *et al.,* 2010).This is in relation to the low water quality (Table 1a) that is below the World Health Norms for lotic aquatic media and thus raises environmental concerns.

There is rapid urbanisation of humans in developing regions and the further stress that is placed on inadequate water supply and sanitation associated with increased human activity such as eutrophication of waterways, results in the increase in oocysts densities. This value are in conformity with the values recorded for the conductivity, TDS, Suspended Solids and the anionic and cationic balance along the streamcourse (Table 1a and 1b). Important sources of *Cryptosporidium* and *Giardia* contamination of surface water are discharges of untreated domestic sewage and agricultural runoffs, an increase in the parasite concentration in surface water is observed

downstream of agricultural areas as in the case of the Mfoundi river basin where the practice of urban agriculture does not take into consideration the physico-chemical and microbiological properties of the water being exploited. This results in an increase in abiotic variables(Table1a, 1b and 2).The high and positive relationship observed in Table 2, could be due to the fact that ,the metropolitan city of Yaounde is not an industrial zone, thus the mechanisms responsible for aquatic ecosystem contamination are basically domestic and anthropogenic. The abiotic variables concomitantly increase downstream , as a result of a high demography and low sanitary conditions available for the population exploiting the aquatic resources.

Table1a. Ecological characteristics of the aquatic ecosystem analysed from Mfoundi River.

1a: Physical parameters.

Sampling points/Parameter	Conductivity (µsiemens/cm)	TDS (mg/L)	pH	Temperature oC	SS (mg/L)
Mfoundi 1	185.22± 1 97.17	92.28 ± 98.72	6.63 ± 0.41	22.42± 1.25	115.55± 205.23
Mfoundi 2	289.65± 42.57	148.69 ± 20.62	6.96 ± 0.19	24.34 ±1.28	284.72± 308.88
Mfoundi 3	267.28± 37.03	133.21 ± 43.66	6.80 ± 0.21	23.71± 1.11	246.60± 29.01

Table 1b. Chemical parameters.

Points/ Parametre	O_2 (mg/L)	CO2 (mg/L)	Ca (mg/L)	Ca+Mg (mg/L)	Mg (mg/L)	Alk (mg/L)	Cl (mg/L)	Oxyd (mg/L)	BOD (mg/L)	Sat	PO4 (mg/L)
Mfoundi 1	2.19±0.6	146.6±60.4	21.4±13.0	108.2± 64.4	12.9±10.7	20.3±16.2	2.1±1.7	13.9±6.5	20.4±12.0	25.7±8.3	4.58±2.2
Mfoundi 2	1.8±0.7	193.5±99.7	38.2±16	191.1±87.3	23.4±14.2	70.2±25.0	5.2±2.9	22.0±13.8	70.7±25.8	21.1±8.1	6.49±1.5
Mfoundi 3	1.89±0.7	163.2±78.4	35.6±17	182.3± 88.8	18.0±14.4	67.4±47.1	4.1±2.0	20.4±10.5	97.5±42.8	23.1±9.2	5.23±1.8

Table 2. Pearson correlation coefficient for the biological and environmental variables

Parameter	Sampling point	Correlation
Oocysts/Cysts	M1/M2/M3	0.888
Physical/Physical	M1/M2	0.940
	M2/M3	0.998
	M1/M3	0.957
Chemical/Chemical	M1/M2	0,956
	M2/M3	0,984
	M1/M2	0,902

The temperature and other environmental variables of the ecosystem can have a synergistic adverse effects on oocysts viability(Jenkins *et al.,* 1998).The glycocalyx external structure impart the oocysts with structural and functional stability under gastrointestinal conditions. Organic matter inhibits microbe transport due to hydrophobic interactions between microbe and grain surfaces that are coated with organic matter: A variety of chemical ;electrostatic sedimentation and interactions and biological factors can play a vital role in oocysts-suspended particles interactions and oocysts-oocysts interactions(Dai and Boll,2003) as presented in Fig. 2-3, Tables 1-2. Due to their small size and varying morphologenesis; these enteropathogens are generally considered to move through watersheds from their source to drinking water reservoirs with little attenuation in their dynamics in the stream channel ,however the transport of oocysts in surface water may be mediated by interactions with suspended sediments and by subsurface filtration and removal in streambed sediments(Hsu *et al*., 2001).The attachment of these parasites to several inorganic and organic sediments under varying water quality conditions may drastically influence the effective in stream settling velocity of *C parvum* oocysts as stipulated by Brush *et al.,* 1999.

According to Brooks *et al.,* 2006, there is a possibility that the oocysts can survive more in the water column than in the sediments. The pathogen sediment interactions play an important role in regulating the concentration of these pathogens in streams and rivers and should be taken into consideration when predicting their fate in the environment (Anya *et al.,* 2010). There is also the possibility of peril faecal contamination of the water system (Wuhip *et al.,* 1994; Ma and Juan, 2010). These protozoan can have the a potential to spread through multiple modes of transmission with a common source outbreak caused by their exposure to contaminated source ,resulting in subsequent prolonged propagation through person to person transmission in the community(Giovanni *et al*., 2006; Amy *et al*., 2010,).

There is a possibility of simultaneous outbreak of these diseases, indicating the significant correlation observed between the oocysts, due probably to their predirection for immunocompromised systems, zoonotic and anthroponotic potentialities, ability to shed very resistant forms and the faecal oral mode of contamination. These waterborne parasites are ubiquitous in the city of Yaounde and thus affect the human population and other animal present in this municipality, due to the fact that routine methods of disinfection such as chlorination and ozonation is rendered difficult due to the small size and the environmental robustness of the transmissive stage of these protozoan .An additional stage of filtration, cleaning and disinfection can reduce the abundance of these oocysts and cysts in the aquatic environment. A calculation of the number of oocysts discharged into surface water through the untreated effluents makes it possible to select the most appropriate preventive measures for source water protection, both on the national and regional scale(Giovanni *et al.,* 2006).This study is a contribution to the quantitative description of the transmission cycle of *Cryptosporidium* spp and *Giardia* spp in surface water in a Metropolitan aquatic ecosystem.

Conclusion

Cryptosporidium spp oocysts and *Giardia* spp cysts have been isolated and then identified in the surface water of Yaounde by the USEPA recommended method 1623.The (oo) cysts are released into the Mfoundi mainstream where they are evacuated and transported to other regions of the hydrosystem in which physical and chemical parameters play a role in their transmission and infectiosity . There is a remarkable increase in the density of the resistant form of the parasite from upstream to downstream indicating an accumulation of the effects of anthropogenic activity and a possible zoonotic contamination of the

ecosystem where they can lead to chronic gastro-intestinal diseases. The modelling of the discharge of these parasitic protozoa into surface water gives an insight into the dispersion of these pathogens in the aquatic ecosystem

References

Amy JP, Alexandria BB, Mathew M, Jennifer D. 2010. Efficacy of waterless hand Hygiene compared with handwashing with soap, A field studying Dar es Salaam, Tanzania. American Journal of Tropical Medecinr, Hygiene, **82(2),** 270-278.

Anya O, Aman DV, Benoit B, Annie C, Romain P, David LB. 2010. A simulation model of waterborne Gastro-Intestinal Disease outbreaks.Description and Initial Evaluation .Amia Annu Symp Proc, 557-561**.**

Arnone RD, Walling JP. 2006. Evaluating *Cryptosporidium* and *Giardia* concentrations in combined sewer overflow. Journal of Water Health **4(2),** 157-65.

Bachelier G. 1959. Etude pédologique des sols de Yaoundé ; contribution à l'étude de la pédologie des sols ferrallitiques. Agronomie Tropicale XIV**, 3**, 279-305.

Brandinosio O. 2006. Waterborne transmission of *Giardia* and *Cryptosporidium* . Parasitologia, 4**8**(1-2): 91-4.

Brush FC, Ghiorse WC, Lynne JA, Parlange J. V. Ghiorse HG. 1999. Transport of *Cryptosporidium parvum* oocysts through saturated columns .Journal of Environ Quality **28**, 809- 815 .

Brooks JD, Davies CM, Hipsey MR, Antenucci P. 2006. Association of *Cryptosporidium* with bovine faecal particles and implications for risk reduction by settling within water supply reservoirs. Journal of Water Health **4(1),** 87-98.

Brush FC, Ghiorse WC, Lynne JA. , Parlange JV, Ghiorse HG. 1999. Transport of *Cryptosporidium parvum* oocysts through saturated columns .Journal of Environ Quality **28**, 809- 815 .

Craun GF, Brunkard JM, Yoder JS, Roberts VA, Carpenter J, Wade T, Calderon RL, Roberts JM, Beach MJ, Roy SL. 2010. Causes of outbreaks associated with drinking water in the United States from 1971-2006, Cinicalo Microbiology Review **23(3),** 507-528.

Dai X, Boll J. 2003. Evaluation of the attachment of *Cryptosporidium parvum* and *Giardia lamblia* to soil particles. Journal of Environmental Quality **32**, 296-304.

Doron M. 2000. Cryptosporidium dans l'environment aquatique.consequence pour les eaux de distribution. (Service National d'information et de documentation sur l'eau. (SNIDE).

Giovanni GD, Betancourt WQ, Joe H, Naomi W, Assadian, Juan P, Flores M, Esaul JL. 2006. Investigation of potential zooanthroponotic transmission of Cryptosporidiosis and Giardiasis through agricultural use of reclaimed wastewater. International Journal of Environmental Health research **16(6),** 1-32.

Harter T, Atwill E, Hou L, Karle B, Tate K. 2008. Developing risk models of *Cryptosporidium* transport in soils from vegetated , tilted soil box experiments. Journal of Environmental Quality **37(1),** 245-58.

Hsu BM, Houang C, Pan JR. 2001. Filtration behaviour of *Giardia* and *Cryptosporidium* .Ionic

strength and pH effects. Water Research **16,** 3777-3782.

Jenkins MB, Bowman DD, Ghiorse WC. 1998. Inactivation of *Cryptosporidium parvum* oocysts by ammonia. Applied Environmental Microbiology **64**, 784-788.

Kabore H, Pascal M, Levallois P, Dery P, Payment P, Lebel G. 2010. A descriptive review of selected non viral enteric illnesses reported in children in Quebec between 1999 and 2006, Canadian Jounal of infectious Diseases and Medical Microbioly **21(2),** 92-98.

Keush GT, Kelly J, Ward H. 1995. Cryptosporidia-who is at risk. Schweizmedwochensihev **125(18),** 899-908.

Ma JF, Juan JB. 2010. News perspectives in monitoring drinking water ,Microbial Quality , International Journal of Environmental Research, Public Health 7(12), 4179-4202

Mok-Young L , Eun-Joo C, Jin-Hyo L, Sun-Hee H, Yong-Sang P. 2011. A ten year survey of Giardia cysts in drinking water supplies of Seoul , The Repubic of Korea, Korean Journal of Parasitology **49(1),** 9-15.

Savioli L, Smith H, Thompson A.. 2006.*Giardia* and *Cryptosporidium* join the 'Neglected Diseases Initiative .Trends in Parasitology **22(5),** 203-208.

Suchel J. 1972. Travaux et documents de géographie tropicale - N° 5 - La répartition des pluies et les régimes pluviométriques au Cameroun. Université du Cameroun / TALENCE, CEGET Centre d'Études de Géographie Tropicale / CNRS Centre National de la Recherche Scientifique, p. 267.

Wuhip T, Silva TM, Newman RD, Garcia LS. 1994. Cryptosporidial and Microsporidial infections in human immunodeficiency virus-infected patients in Northeastern Brazil. Journal of Infectious Diseases **170(2),** 494-497.