MICROBIOLOGICAL CONTAMINATION OF GROUNDWATER BY CRYPTOSPORIDIUM OOCYSTS IN HAITI. HEALTH RISK ASSESSMENT FOR POPULATION

CONTAMINATION MICROBIOLOGIQUE DES EAUX SOUTERRAINES PAR LES OOCYSTES DE CRYPTOSPORIDIUM EN HAÏTI. EVALUATION DES RISQUES POUR LA SANTÉ DE LA POPULATION

CONTAMINACIÓN MICROBIOLÓGICA DE LAS AGUAS SUBTERRÁNEAS POR LOS OOCITOS DE CRYPTOSPORIDIUM EN HAITÍ. EVALUACIÓN DE LOS RIESGOS PARA LA SALUD DE LA POBLACIÓN.

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Abstract

Contamination of natural aquatic ecosystems by Cryptosporidium is a major environmental and human health issue. In Haiti, environmental *Cryptosporidium* oocysts pollution has been well documented by previous studies conducted in several cities of the country. In groundwater from Les Cayes of Haiti, significant concentrations from 1 to 989 oocysts in 100 liters of filtered water were calculated. Results of these studies revealed high level of *Cryptosporidium* oocysts pollution in surface water and groundwater. Therefore, regarding cryptosporidiosis, contaminated water resources constitute a real sanitary risk mainly for children and immunocompromised individuals. So, it is necessary to assess the biological risk for populations served by those polluted water resources. The aim of this study is to present: (i) the steps of a procedure intended to evaluate risks to human health linked to the consumption of water from groundwater; and (ii) the results of its application on groundwater from Les Cayes, city located in southwestern Haiti. The procedure is based on a scenario that describes the existence of an uncontrolled landfill leachate which are neither collected nor treated. The refuse has a close contact with the soil making easy the transfer of various pollutants from the surface to groundwater. Moreover, latrines and septic tanks are often discharged into the unsaturated zone of the geological matrix are also retained. Risk estimation was calculated for two groups in the exposed population: immunocompetent and immunocompromised individuals. As expected, the study revealed a high risk for individuals in the immunocompromised group.

Key words: Groundwater, microbiological contamination, health, biological hazards, risk assessment, Cryptosporidium sp.

Resumé

La contamination des écosystèmes aquatiques naturels par *Cryptospridium parvum* constitue une véritable préoccupation de santé humaine et environnementale principalement dans les pays en développement. En Haïti, des oocytes de cryptosporidies ont été retrouvés dans plusieurs villes du pays dans les eaux de surface et dans les eaux destinées à la consommation humaine. Dans les eaux souterraines Les Cayes d'Haïti, des concentrations significatives de 1 à 989 oocystes dans 100 litres d'eau filtrée ont été déterminées. Les résultats de ces études ont révélé un niveau élevé de pollution par des oocystes de *Cryptosporidium* dans les eaux de surface et des eaux souterraines. Par conséquent, les ressources en eau contaminés par des oocystes constituent un véritable risque sanitaire principalement pour les enfants et les personnes immunodéprimées. Ainsi, il apparaît nécessaire d'évaluer le risque biologique pour les populations desservies par ces ressources en eau polluées. Le but de cette étude est de présenter: (i) les étapes d'une procédure destinée à évaluer les risques pour la santé humaine liés à la consommation d'eau provenant des eaux souterraines; et (ii) les résultats de l'application de cette procédure sur les eaux souterraines Les Cayes, ville située dans le sud-ouest Haïti. La procédure est basée sur un scénario qui décrit l'existence d'une décharge sauvage où les lixiviats ne sont ni collectés, ni traités. Les déchets sont directement en contact avec le sol et suivent les mécanismes de transfert vers la nappe. En outre, les latrines et fosses septiques, rejetant leurs effluents dans la zone non saturée de la matrice géologique, sont également retenues. L'estimation du risque a été calculée pour deux groupes dans la population exposée: immunodéprimé et immunocompétent. Il apparaît comme attendu un risque élevé pour les immunodéprimés.

Mots clés: Eaux souterraines, contamination microbiologique, santé, risques biologiques, évaluation des *risques, Cryptosporidium spp.*

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Resumen

La contaminación de los ecosistemas acuáticos naturales por *Cryptospridium parvum* constituye una verdadera preocupación de salud humana e ambiental principalmente en los países en desarrollo. En Haití, los oocitos de cryptosporidios se han encontrado en muchas ciudades del país en las aguas de superficie y las aguas destinadas al consumo humano. En las aguas subterráneas en Les Cayes de Haití, concentraciones significativas de 1 a 989 oocitos en 100 litros de agua filtrada han sido determinadas. Los resultados de estos estudios han revelado un nivel elevado de polución por oocitos de *Cryptosporidium* en las aguas de superficie y aguas subterráneas. Por ende, los recursos en agua contaminados por oocitos constituyen un verdadero riesgo sanitario para los niños y las personas inmunodeprimidas. Así, se hace necesario evaluar el riesgo biológico para las poblaciones que se abastecen de estos recursos en agua contaminados. El objetivo de este estudio es presentar: (i) las etapas de un procedimiento destinado a evaluar los riesgos para la salud humana ligados al consumo de agua proveniente de las aguas subterráneas; et (ii) los resultados de la aplicación de este procedimiento en las aguas subterráneas en Les Cayes, ciudad situada en el suroeste de Haití. El procedimiento está basado en un escenario que describe la existencia de una descarga violenta en donde los lixiviados no son ni recolectados ni tratados. Las basuras están directamente en contacto con el suelo y siguen los mecanismos de transferencia hacia la capa de agua subterránea. Además, las letrinas y fosas sépticas, rechazando sus efluentes en la zona no saturada de la matriz geológica, están igualmente retenidas. La estimación del riesgo ha sido calculada para dos grupos en la población expuesta: inmunodeprimida y immunocompetente. Este aparece como un riesgo elevado para los inmunodeprimidos.

Palabras claves: Aguas subterráneas, contaminación microbiológica, salud, riesgos biológicos, evaluación de los riesgos, Cryptosporidium spp.

1. INTRODUCTION

Contamination of water resources by *Cryptosporidium* oocysts is a serious public health issue (Suzuki and Takida, 2015). Indeed, *Cryptosporidium,* an extremely virulent microorganism, is persistent in the environment and resistance to chemical disinfection has made this protozoan parasite one of the critical pathogens for the drinking water industry (WHO, 2009). Some species infect humans (Liu, 2012) and animals (Hong et al., 2014). They cause cryptosporidiosis disease and mild to severe diarrhea, dehydration, stomach cramps, and/or a slight fever from waterborne species (Agulló-Barceló et al., 2012). The disease is transmitted in feces by humans and other animals as an oocyst, which has a hard, environmentally resistant shell for protection (Messner et Berger, 2016). Because of it occurrence in groundwater, public water supplies (Balthazard-Accou et al. 2010), and surface water (Rose et al. 1991; Lechevallier et al. 1991a), many cryptosporidial enteritis outbreaks have been reported (MacKenzie et al. 1994; Smith and Rose 1998; Widerström et al 2014). Pathogen infection risk targets are central to some drinking (or other) water exposure evaluations (Sinclair et al., 2015; O'Toole et al., 2015). Both humans and animals may be exposed to *Cryptosporidium* through consumption of contaminated water and food as well as by direct contact with contaminated soils and infected hosts (Fayer, 2004).

The presence of oocysts in natural aquatic environment and drinking water brings a biological hazard, which is linked to the existence of the dangerous aspects of this particle (Rivière, 1998); and may generate biological risks of *Cryptosporidium* for human health. Otherwise, biological, environmental, climatic and community habits are involved in the potential risk factors for waterborne transmission of cryptosporidiosis (Rose et al. 2002). The deficit of policy in urban planning can be also taken as a risk factor for diseases related to *Cryptosporidium*. Indeed, land use activities contributing feces for example show that waters receiving cattle and sewage discharges have 10-100-fold greater concentrations of oocysts (Bagley et al., 1998). In this case, transportation through soil has usually been considered an insignificant pathway because soil is generally assumed to be an effective filter inhibiting the transport of different pathogens (Petersen et al. 2012). For colloid-sized *Cryptosporidium* oocysts the fate and transport processes depend much on the soil physical and chemical properties (Peng et al. 2011).

In Haiti, the presence of *Cryptosporidium* oocysts in soils (Bathazard-Accou et al. 2014), in surface and groundwater (Balthazard-Accou et al. 2009, Brasseur et al. 2011), and its transport from soils to groundwater have been studied (Balthazard-Accou, 2011). Several factors could be responsible for groundwater exposure to *Cryptosporidium* oocysts especially the discharge of urban effluents into rivers without any prior treatment and the existence of latrines and septic tanks equipped with infiltration wells in a high-risk flood area (Balthazard-Accou et al., 2014).

Furthermore, *Cryptosporidium* is responsible for 17% of cases of acute diarrhea observed in infants under the age of 2 (Pape et al., 1987). In Port-au-Prince districts, where water contains *Cryptosporidium* oocysts, the estimated risk of infection is between $1x10^2$ and $5x10^2$ for the immunocompetent population; for the immunodepressed population, this value varies from $1x10^2$ to $97x10^2$, depending on the oocyst load in the consumed water (Bras et al., 2007). However, this microbiological risk estimated for *Cryptosporidium* only focused on a few aquatic ecosystems in Port-au-Prince. The aim of this study is to present: (i) the steps of a procedure intended to evaluate risks to human health linked to the consumption of water from groundwater; and (ii) the results of its application on groundwater from Les Cayes.

2. METHODOLOGY

2.1 General approach of health risks evaluation

The National Research Council (1983) defines the assessment of risks as the activity that evaluates the toxic properties of a chemical product and the conditions of human exposure to this product, in order to observe the reality of human exposure and characterize the nature of the effects that may result. The general approach of health risk assessment is based on four steps: identifying the hazard, studying the dose-response relationship, estimating exposure and characterizing the risk (NRC, 1983).

In the field of chemical risks, methodological guides refer to the available tools, whether it is models or databases of toxicological and physico-chemical data. On the contrary, the biological risk has many characteristics that prevent a simple transposition of the methodology from the chemical to the biological field area. The difference in methodology between the estimate of a chemical risk and that of a microbiological risk lies in the identification of dose-response functions and particularly in the choice of the model of dose-response relationship (Gofti, 1999). Human, animal, and environmental reservoirs are notoriously difficult to control and quantify (Zmirou-Navier et al., 2006).

2.2. Presentation of study site

Les Cayes is a city located in Haiti's Southern region. Its population is currently 137,952 habitants (IHSI, 2009). The city is located at 18°34'00'' Northern Latitude and 72°21'00" West Longitude on the Caribbean coast, on a coastal plain with high rainfall (over 2,000 mm/yr), The average temperature varies from 24°C to 28°C. There are two rainy seasons: from April to May and August to October.

There are several types of groundwater, among them: unconfined alluvial aquifers, karst aquifers, giving rise to a variety of resurgences and flows (PNUD, 1991a). The groundwater resources are used for drinking water. From an ecological point of view, they represent a considerable amount of reserve water and play a major role in the feeding of many lakes and rivers. The mode of supply is from groundwater extraction with the installation of wells and boreholes, and spring catchments. Distribution is via private networks and connections, and public standpipes. According to Balthazard-Accou (2011), the municipal water system is supplied by two wells with a flow rate of 66 L/s and an average production of about 10,134 m^3/day.

In addition, due to the topography of this coastal city, the existing latrines are easily in contact with groundwater; especially during the rainy season, facilitating the movement of microorganisms. Moreover, the city is very sensitive to flooding. A brief description of the urban environment of the city is presented in the scenario (Figure 1) developed for the implementation of this biological risk assessment.

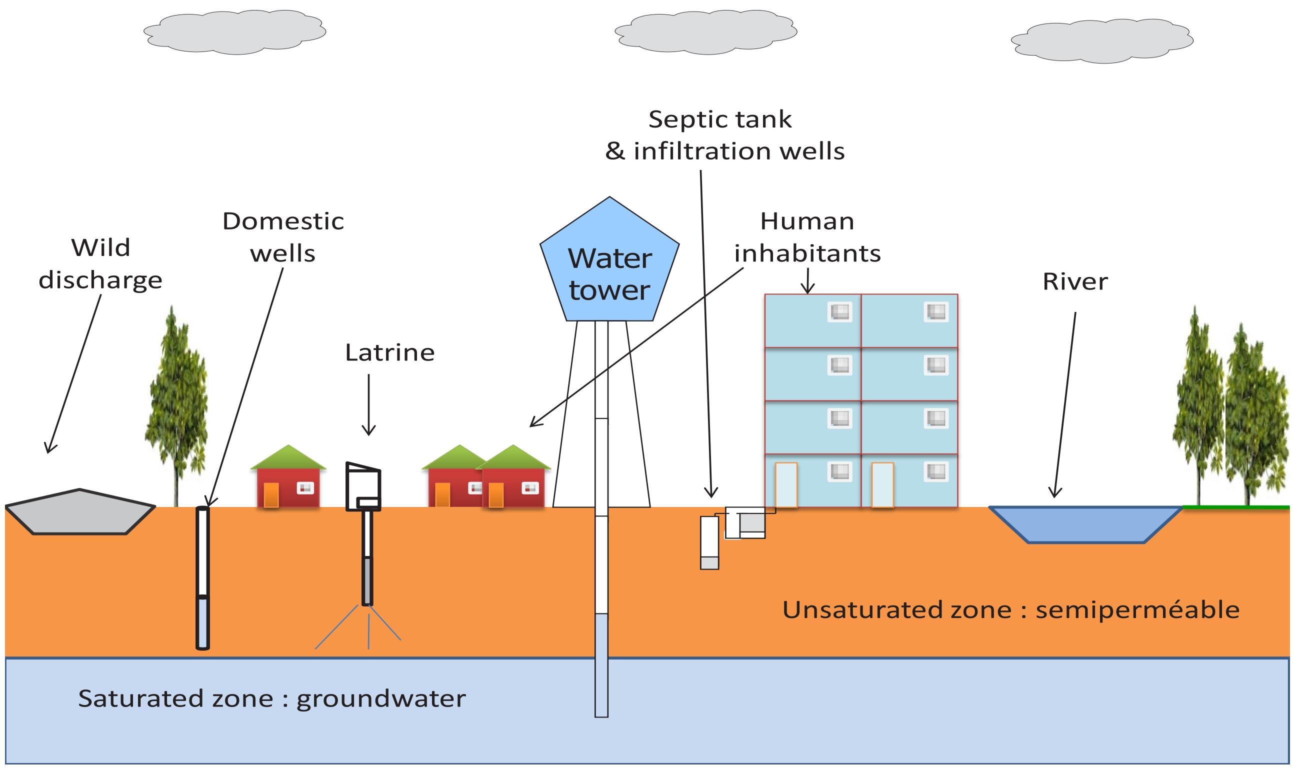

Figure 1. Graphic representation of the scenario studied

The scenario highlights the existence of a dumpsite where leachates are not collected or treated. They are in direct contact with the soil and they follow the transfer mechanisms toward the groundwater. Latrines and septic tanks, discharging into the unsaturated geological matrix area, are also noted. Other utilities such as boreholes and wells feeding a family or group of families with water, an urban water supply and an individual sanitation network are also presented in this scenario (Figure 1).

The scenario reproduces the supply mode of drinking water and wastewater management in Les Cayes. Percolation of leachates from uncontrolled discharge, the hydraulic operation of the effluents generated by latrines and septic tanks, contamination of surface water and the interactions between these aquatic ecosystems and groundwater reflect the existence of a risk to human health that may result from the ingestion of water from the aquifer or surface water. The very use of the aquifer for water supply, taken in the particular context of the scenario observed, can be an important source of distribution of infectious waterborne diseases in the study area.

In order to avoid initially conducting a major campaign of sampling in different emission sources of oocysts toward the groundwater, we have introduced in the analysis plan an initial step consisting in working only on water from the groundwater and that is used by people. This phase aims at detecting *Cryptosporidium* oocysts during the two rainy seasons of the year and comparing the different results obtained in water from the groundwater for pathogen selected within the number zero to 100 liters of water.

For any number of oocysts less than 1 per 100 liters of water intended for human consumption, the flowchart showcases the absence of *Cryptosporidium*, which in turn justifies the absence of contamination, thus there is no hazard (risk) for the population. However, this type of procedure recommends the implementation of a microbiological surveillance program consisting of periodic characterization of *Cryptosporidium* oocysts in the water from the groundwater. In contrast, for any number of oocysts greater than or equal to 1 per 100 liters of water intended for human consumption, the approach recommends the next steps of the evaluation of biological risks to consumer health (Figure 2).

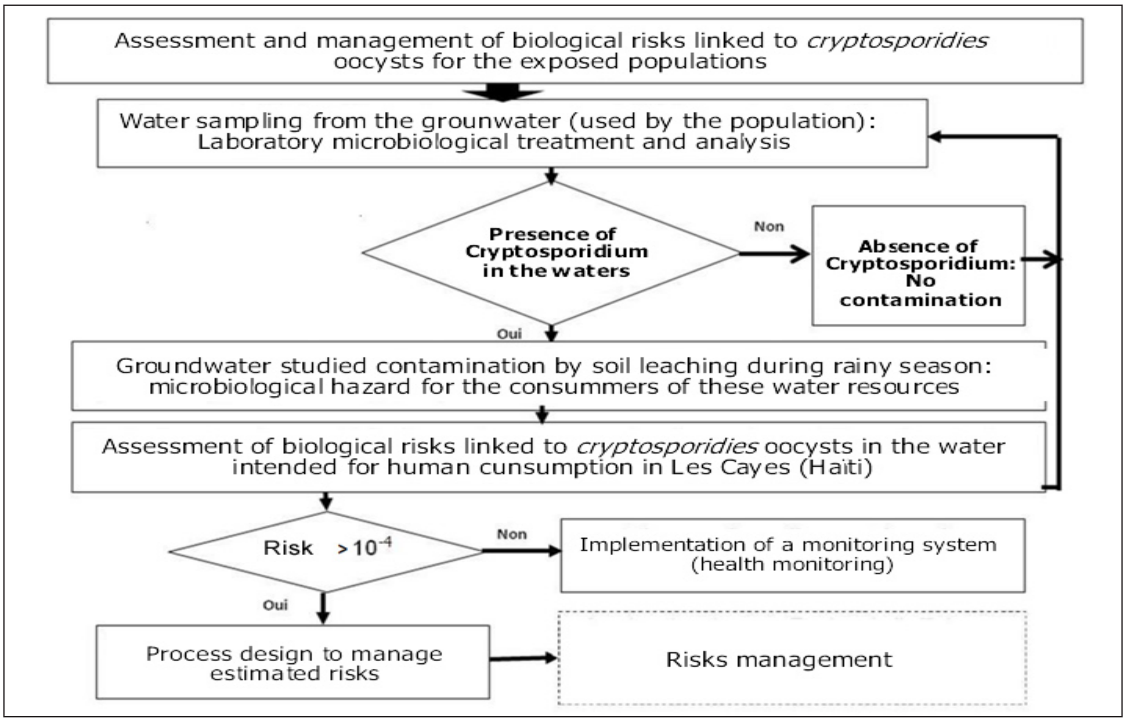

Figure 2. Flowchart developed for the implementation of the biological risk assessment due to the presence of *Cryptosporidium* oocysts

2.3. Identification of danger

For the microbiological risk assessment of *Cryptosporidium* in drinking water, an approach based on the analysis of the numerous factors responsible for potential exposure of Les Cayes groundwater to biological contaminants has been developed. Among these factors : i) cattles wandering freely in the city leading to a permanent and significant spread of their feces laden with bacteria, viruses and other parasites into the groundwater; ii) discharge of urban effluents into rivers without any prior treatment; iii) the existence of latrines and septic tanks equipped with infiltration wells in a high-risk flood area; iv) the disposal of sludge from latrines and septic tanks on the floor of an alluvial formation; v) the existence of illegal landfills in an alluvial formation with unprotected geotextile, with no leachate collection,etc. In this study, *Cryptosporidium* has been identified as the single agent potentially most dangerous for people consuming water from the groundwater of Les Cayes. Indeed, previous studies show the presence of *Cryptosporidium* in these water resources (Balthazard-Accou et al. 2009).

2.3.1. Cryptosporidium

It is an indicator or marker of faecal pollution in water. Infectious diseases are mainly transmitted by human and animal excreta, particularly faeces. Contamination can occur via diseased persons and carriers of germs in the community, who contaminate the water supply with pathogenic microorganisms. The consumption of this water can lead to infection and represents a biological hazard to the exposed population exposed. The presence of oocysts in water is an important risk factor for human health, especially for the most vulnerable groups (Craun et al. 2005; Coupe et al. 2006; Raccurt, 2006).

2.4. Exposure assessment

In the particular context of Haitian cities, where weak urban services contribute significantly to the pollution of groundwater, it seems that health risk assessment (biological and / or chemical) studies, must borrow from the conventional ecological risk assessment the use of the concept of the "conceptual model". This model could better help appreciate the dual role played by groundwater, namely: (i) the target surface pollution, and (ii) the source of tap water for human consumption. Figure 3 illustrates the conceptual model.

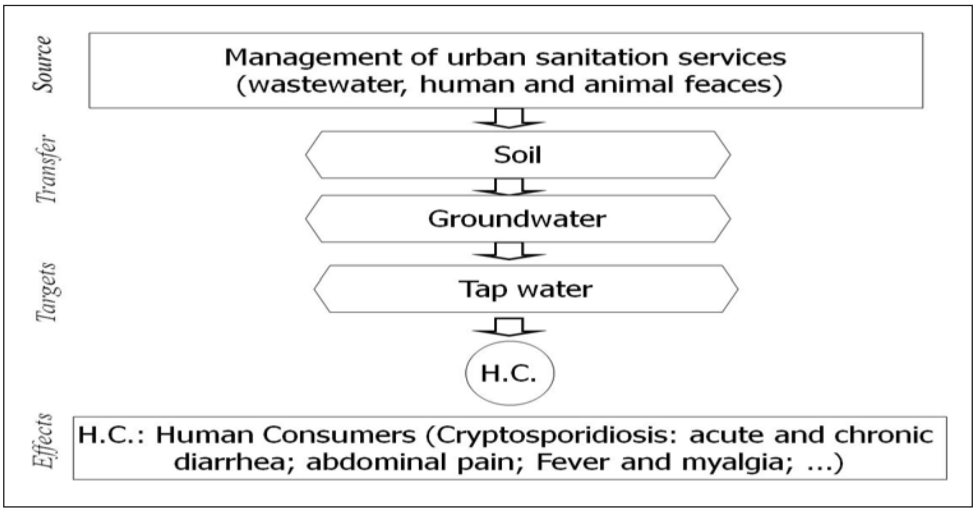

Figure 3. Conceptual model studied

In this study, the term "transfer" is represented by the groundwater. It is therefore chosen to study the impact of "pollution on the surface" on groundwater quality. Not taking into account that surface water does not mean that it is of lesser importance in the contamination of groundwater. We believe that the phenomena of microbiological contamination of groundwater through hydrological mechanisms that govern the interactions between surface water and groundwater will require further studies.

However, exposure assessment also aims to investigate the potential contamination by *Cryptosporidium* oocysts of water from groundwater beneath the site. For this purpose, data on population exposure, routes of exposure, concentrations and the frequency or distribution of *Cryptosporidium* oocysts in space and in time, the duration of exposure, the quantitative estimation of human exposure and also the transport of these oocysts to the groundwater are required (Haas et al. 1999).

3. MATERIALS AND METHODS

Collection of water samples

Between September 2007 and February 2010, a total of five sampling campaigns of water samples were performed, including three rainy seasons in 2007-2009 and two droughts in 2009-2010, specifically between the end of the long rainy season and the beginning of the long dry season. During these campaigns, 25 samples were collected on 5 sampling points (CA03, CA05, CA07, CA09, CA13) used for drinking water. Turbidity, pH, electric conductivity and temperature were performed in situ. Samples for physico-chemical analyses were placed in clean polyethylene bottles. All samples were collected by the instant manual sampling method. The pH was measured using a multimeter HACH HQ40d field case 58258-00. The turbidity of the samples was measured using a 2100P 46500-00 Hach turbidimeter. A multimeter HACH HQ40d field case

58258-00 was used to measure electric conductivity and temperature.

To minimize cross-contamination in the field, new water sampling equipments (bucket, tumbler and funnel) were used at each sample site. A sample of at least 100 L of water was collected and immediately filtered using a polyethersulphone capsule (Environchek, Pall Gelman, Saint Germain en Laye, France). Capsules were stored at 4°C until the elution step.

Purification of Cryptosporidium oocysts

Capsules were processed respectively the method of concentration and counting AFNOR NFT90-455 (AFNOR, 2001). Briefly, capsule filters were rinsed with 240 ml of a detergent elution buffer (phosphate-buffered Saline, pH 7.4 with 0.1% (v/v) Tween 80). Specimens were concentrated by centrifugation at 3500g for 30 min and at a temperature of 4°C. The final sediment was suspended in double-distilled water with a final volume of around 5 ml. Any *Cryptosporidium* oocysts present were then purified using immunomagnetic beads coated with anti-*Cryptosporidium* monoclonal antibody (Dynabeads, Dynal, Norvège) according to the manufacturer's instructions.

Detection and counting of Cryptosporidium oocysts

Twenty microliters of suspension derived from the IMS procedure were placed on a glass slide and dried at room temperature. Slides were fixed in cold acetone (20°C) for 10 min and were then incubated for 30 min at 37°C in a humid chamber with a 1:10 final dilution of a fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (MAb) directed against a *Cryptosporidium* wall antigen, which was selected because of its lack of cross-reactivity with other microorganisms (FITC-Cow MAb, Monofluokit *Cryptosporidium*, Bio-Rad, Marnes la Coquette, France). Slides were rinsed with PBS (pH 7.4) before applying coverslips. The entire smear of each slide was examined using an epifluorescent microscope (UVexcitation at 490 nm, emission 456 nm; BX41, Olympus) and oocysts were counted. A positive control slide was used to ensure IFA results. The number of oocysts was expressed per 100L of filtered water.

3.1. Risk characterization

3.1.1. Definition of populations exposed by studying the type of exposure identified

The available information on the performance of the public service of water supply does not allow us to exactly define the exposed population. For the purposes of this study, a total population served by the public water supply service and four small family and / or community systems, was estimated through the criteria defined by OPS/OMS and BID (1996) for public water supply in the urban areas of Haiti.

The computation criteria adopted for estimating individuals being served with drinking water in the urban area are (OPS/OMS and BID, 1996) : (i) the number of people served by regular private connection is: ... 14 and, (ii) the number of persons served by public fountain is ... 500. In this study, a coefficient of 14 is applied to 986 connections served by the SNEP, and another one of 500 to the 4 small community water supply systems. In general, the total population considered in this work is 15804 persons exposed (children and adults of both sexes). The main exposure path identified and studied was the consumption of drinking water.

In the general approach of health risk assessment of drinking water, total consumption of 2 liters of water per day for adults and 0.75 liter per day for children is often adopted to calculate the average daily dose (Fawell and Young, 1999). Body weight of 70 kg and 10 kg respectively were attributed to adults and children under 10 years.

In this study, the total exposed population was divided into two major types: immunocompetent and immunocompromised. Each of these types has 2 classes: children 0-14 years and adults 14 years and older. The exposed population is thus distributed as follows:

- immunocompetent children aged under 14;
- immunocompromised children aged under 14 years;
- immunocompetent adults aged 14 years and older;
- immunocompromised adults aged 14 years and older.

Information coming from: (i) the general census of the population and housing for the year 2003, produced by the Haitian Institute of Statistics and Informatics, and (ii) study on the HIV seroprevalence in Haiti for the period 2007-2012 (USAID, 2007), has been used to distribute the 4 target groups on the study site.

For each sampling point, 40% of the population served is represented by children under 14 years, and 60% of the population is aged 14 and over. A total number of 6322 children under 14 years, and 9,482 people of 14 years and over constitute the exposed population. In Table 1, the "Infected population in %", were applied to each point studied in this work. Table 2 shows the distribution numbers of immunocompetent and immunocompromised for each of the age groups considered.

Table 1. Estimate of the immunocompromised population in the Commune of Les Cayes

Site	"Less than 14" population	"14 and more" population	Total	References
Département du Sud	254,940	429,862	684,802	IHSI, 2003
HIV infected population	630	8,272	8,902	USAID, 2007
Infected population in %	0.25	1.92	1.3	
Commune of les Cayes	55,342	82,610	13,7952	IHSI, 2009
Population distribution in %	40	60	100	
Infected population in %	0.25	1.92	1.3	
HIV infected population	138	1,586	1,724	

Table 2. Estimated populations of the sites under study

Site	"Less than 14 years old" population		"14 years old and more" population	
	Immunocompromised	Immunocompetent	Immunocompromised	Immunocompetent
Ca03	1	199	6	294
Ca05	14	5,508	159	8,123
Ca07	1	199	6	294
Ca09	1	199	6	294
Ca13	1	199	6	294
Total	18	6,304	183	9,299

The infected population rate has been maintained to estimate the number of people infected with HIV in the town of Les Cayes. Certain assumptions were made: i) the weight of each of the age groups in the total population has not changed in terms of time (between 2003 and 2009) and spatial (between the various municipalities), ii) the seroprevalence is spread evenly over the entire department.

3.1.2. Biohazard

According to the "Exponential" model (Haas et al. 1999), which assumes independence of action of microorganisms during the initiation phase of an infection, each microorganism has a nonzero chance to cause the infection by itself. The amount of ingested microorganisms does not affect the probability of infection. It all depends on the relationship between the actual number of surviving organisms and the likelihood of colonization of the host. According to this model, the probability P of being infected by the ingestion of a dose of pathogenic agents is expressed mathematically by the following equation:

$$P_{inf} = 1 - \exp(-rD) \quad \text{With } D = \mu.v \qquad \text{Eq.1}$$

This exponential model provides a mathematical description of the infection probabilities distribution.

P_{inf}, represents the probability of infection of an individual exposed to a dose *D* of microorganisms; *V,* is the unique volume of fluid consumed;

μ, the number of organisms per liter in volume consumed;

r, the fraction of surviving organisms ingested to cause infection.

This model seems to best describe the dose-response relationship of C. *parvum*.

The dose-response relationship admits the hypothesis of a lack of synergy between the oocysts; this may reflect that the risk associated with the consumption of 1 oocyst all 365 days of the year is exactly equal, by using the exponential dose-response law, to the risk associated with the consumption of, on any given day, 365 oocysts (AFSSA, 2002). To assess the annual risk of infection, it suffices to estimate the number of oocysts ingested by an individual during a year. The risk of a year of exposure is estimated by the expression (Haas et al 1999.):

$$P_{ann} = 1 - \exp(-rD_{365}) \qquad \text{Eq.2}$$

D, average dose of oocysts ingested in 1 day or

$$P_{ann} = 1 - (1 - P_{inf})^{365}$$

D is the total number of oocysts ingested during the year; P_{ann}, represents the probability of infection per year.

At the risk characterization level, we have also made clear the assumptions retained at each stage of the process and we justified them. These are surrounded by uncertainties that lie at the level of the assumptions retained in terms of dispersion of the pathogenic micro-organisms and the exposure of an individual or population, non representative sampling issues, measurement error, inadequate data (use of generic data), spatial variability, temporal and interindividual.

Therefore, one must collect all the uncertainties. This allows for a confidence index in the final result. The Bootstrap method was applied. Also called Bootstrap Simulation, it involves generating repeated data using a re-sampling (Efron and Tibshirani, 1993). The methodology of estimating the uncertainty involves generating subsets of data, based on a random sampling, replacing them gradually as the data are sampled. Thanks to such re-sampling, each data can be shown in an experiment. The model can then be adjusted to each replicate data sets, thereby generating a random sample of the parameter estimates, one for each repetition. These estimates can then be used to establish a confidence interval for the dose-response relationship or to assess the uncertainty for a given dose.

4. RESULTS AND DISCUSSION

4.1. Results of physicochemical analyzes and of Cryptosporidium oocysts in groundwater

The results of the physicochemical and microbiological analyses of groundwater are summarized in Tables 3 and 4.

Table 3. Results obtained during the rainy season 2007-2009

Site	pH	Turbidity (UNT)	Conductivity (µs/cm)	T°C	Number of oocysts/100 L
Ca03	7,41 [7,36 - 7,46]	0,28 [0,27 - 0,28]	340 [315 - 365]	29,25 [28 - 30,25]	3,33 [0 - 10]
Ca05	7,50 [7,50 - 7,51]	0,37 [0,24 - 0,50]	453 [446 - 460]	28,5 [28,4 – 28,6]	6,33 [5 - 9]
Ca07	7,46 [7,42 - 7,50]	0,79 [0,49 - 1,09]	342 [321 - 362]	25,7 [25,0 – 26,3]	1,33 [2 - 2]
Ca09	7,13 [7,13 - 7,13]	0,74 [0,70 - 0,77]	453 [425 - 480]	27,9 [27,8 – 28,0]	34,33 [1 – 100]
Ca13	7,44 [7,42 - 7,45]	0,38 [0,37 - 0,39]	361 [339 - 383]	28,4 [28,0 – 28,5]	9,26 [3 - 23]

Table 4. Results obtained during the dry season 2009-2010

Site	pH	Turbidity (UNT)	Conductivity (µs/cm)	T°C	Number of oocysts/100 L
Ca03	7,58 [7,50 - 7,65]	0,93 [0,53 - 1,33]	337 [330 - 344]	25,7[25,5 - 25,9]	2 [0 - 4]
Ca05	7,45 [7,40 - 7,50]	0,23 [0,22 - 0,23]	258 [251 - 264]	26,2 [25,6 – 26,7]	121,5 [3 - 240]
Ca07	7,53 [7,48 - 7,57]	0,29 [0,20 - 0,38]	338,5 [338 - 339]	25,05[24,7 – 25,4]	506,5 [24 - 989]
Ca09	7,30 [7,29 - 7,32]	1,15 [0,59 - 1,71]	258,5 [257 - 260]	26,7 [25,6 – 27,8]	0 [0 – 0]
Ca13	7,49 [7,46 - 7,52]	0,26 [0,14 - 0,38]	314,0 [310 - 318]	25,8 [25,5 – 26,1]	18 [0 - 36]

During the rainy and dry seasons the pH values measured on the sites show an average around 7.13 to 7.65, indicating that the drilling water has a slightly basic trend. All results recorded for this parameter are included within the ranges proposed by OMS (1994) for drinking water. Similarly, the conductivity values vary from 257 to 480 $\mu S.cm^{-1}$. Some are greater than the threshold value (400 $\mu S.cm^{-1}$) for the conductivity of the water intended for human consumption (Sigg et al., 2000).

The values for turbidity are in the range of the intervals found in the literature (Lechevalier et al 1991b; Di Giorgio et al 2002; Simmons et al 2001). From one sample to another, the measured values present a variation from 0.14 to 1.71 NTU. The maximum values obtained are above the threshold value of 1 NTU imposed by international guidelines (USEPA, 1999). It seems that high levels of turbidity (more than 1 NTU) and rapid changes in this parameter (from 0.5 NTU in a few hours) are indications of a possible contamination of groundwater by these microorganisms. A similar relationship was highlighted by Laing (2002) and reminds a value of 0.3 NTU should not be exceeded; otherwise the risk associated with the presence of oocysts is more important.

The numbers of oocysts detected during the dry and rainy seasons of the years 2007-2010 for the five sites selected for drinking water from the groundwater are shown in Tables 3 and 4. The lowest concentrations were recorded during the months of August - September, which is the period of the rainy season (Table 3). Other concentrations, the highest in oocysts, were recorded during the months of December to February, which is the (Table 4). Peak oocyst concentrations were observed for both seasons. In the absence of epidemiological data on the prevalence of cryptosporidiosis in Les

Cayes during the 2 seasons, it should be interesting in the future to correlate the infections caused by *Cryptosporidium* at the city level with the seasonal concentrations of oocysts detected in drinking water. Newman (1994) reported the observation of cases of cryptosporidioses in tropical developing regions during the hot and humid season.

The information provided in Tables 3 and 4 allows to hold a number of oocysts between 0 and 989 in the samples studied. With reference to the flow chart developed for the implementation of the biological risk assessment, especially in the phase on the number of oocysts in water used for human consumption, the need to move to the estimation of biological risks to the consumer health becomes important in order to complete this study.

4.2. Characterization of Risks for human health

Table 5 shows the concentration of oocysts in the different sites and the number *of oocysts found in 1 liter of water consumed*

Table 5. Concentration of oocysts measured from the different sites and the number of oocysts per liter of water consumed

Site	Sep 2007 (P)		Aug 2008 (P)		Aug 2009 (P)		Dec 2009 (S)		Fev 2010 (S)	
	C_i	C_f	C_i	C_f	C_i	C_f	C_i	C_f	C_i	C_f
Ca03	0	0	10	0,04	0	0	4	0,016	0	0
Ca05	9	0,036	5	0,02	5	0,02	3	0,012	240	0,96
Ca07	0	0	2	0,008	2	0,008	989	3,956	24	0,096
Ca09	100	0,4	2	0,008	1	0,004	0	0	0	0
Ca13	23	0,092	3	0,012	3	0,0112	36	0,114	0	0

Ci : Concentration of oocysts (/100 L) in the different sites; P: Rainy
Cf : Concentration of oocysts found in 1 liter of water consumed; S: Dry

The method (AFNOR T90-455-NF) advocating the immunofluorescence technique was used for the identification of *Cryptosporidium*. However, it is reported in the literature that there is uncertainty in relation to the method (Drozd, 1996). In order to make the results more viable, AFSSA (2002) estimates the yield of the analysis of oocysts at 40% for distribution water. As part of this study, this yield was applied to estimate the number of oocysts in 100 liters of water, with the assumption that all are viable and potentially infectious. Table 6 shows the yields for the analysis and the number of oocysts per liter of water consumed.

For different classes of the population, the average probability for daily and annual infections was estimated from information reported in the literature on the infectious dose of cryptosporidiosis (DuPont et al., 1995; AFSSA, 2002; Pouillot et al., 2004). Eq.1 and Eq.2 equations were used to calculate the average probability for daily and annual infection.

The main results for the immunocompetent population of 14 years and over are summarized in Table 6.

Table 6. Probability of infection and number of expected diseases cases for immunocompetent and Immunocompromised Population 14 years and over

	Immunocompetent				Immunocompromised			
	Individual infection probability		Population and expected diseases cases		Individual infection probability		Population and expected diseases cases	
Site	Daily	Annual	Population	Diseases cases	Daily	Annual	Population	Diseases cases
Ca03	0,0009	0,03	294	4	0,008	0,94	6	6
Ca05	0,002	0,48	8123	1560	0,11	1	159	159
Ca07	0,006	0,9	294	106	0,19	1	6	6
Ca09	0,006	0,2	294	24	0,05	1	6	6
Ca13	0,004	0,13	294	15	0,03	1	6	6

The portion of the *immunocompetent population aged 14 years and over* consuming water from the site CA03 is exposed to a daily infection probability of 0.0009 and an annual infection probability estimated at 0.03. The site CA05 for its part has an average probability of daily infection of 0.002 while the average probability for annual infection was estimated to be 0.48. As for the CA07, with an average probability of daily infection estimated at 0.006, the average probability of infection over a year is at 0.9. However, the probability of infection for site CA09 was 0.006 and it was estimated to be at 0.2 on an annual basis. The estimate of the probability of infection related to the contamination of water intended for human consumption by *Cryptosporidium* oocysts for site CA13 showed it is approaching 0.004 and while it amounts to 0.13 on an annual basis.

In Table 6, are summarized the main results for *the immunocompromised population 14 years old and over*. For the age group referred to above, immunocompromised and consuming water from the site CA03, the daily probability was 0.008 while it was 0.94 on an annual basis. These values are respectively measured at 0.11 and 1 (that is to say 100%) for the site CA05. They are, in the same order, estimated at 0.19% and at 100% in the site CA07, at 0.05 and at 1 on the site CA09. Substantially following the trend, the probability of infection related to contamination of water intended for human consumption by *Cryptosporidium* oocysts for site CA13 were respectively 0.03 and 1.

Table 7. Probability of infection and number of expected diseases cases for immunocompetent and Immunocompromised Population under 14 years

	Immunocompetent				Immunocompromised			
	Individual infection probability		Population and expected diseases cases		Individual infection probability		Population and expected diseases cases	
Site	Daily	Annual	Population	Diseases cases	Daily	Annual	Population	Diseases cases
Ca03	0,0003	0,001	199	0	0,08	0,95	1	1
Ca05	0,0007	0,21	5508	463	0,05	1	14	14
Ca07	0,002	0,59	199	47	0,14	1	1	1
Ca09	0,002	0,08	199	6	0,02	1	1	1
Ca13	0,001	0,05	199	4	0,01	0,98	1	1

The results for the immunocompromised population of 0-14 years are much higher than those obtained for the immunocompetent population**.** To illustrate, the immunocompromised population is exposed to a daily infection of 0.01 to 0.14 and an average probability of annual infection of 0.95 to 1 from the different sites.

The results from the estimates of the probabilities of infection for the immunocompromised populations categories are comparable between the different sites and much larger than those estimated for the immunocompetent populations. The average probability of infection over a year for some sites is between 0.95 and 1, that is to say, the exposed populations had 95 to 100% of a chance of being infected. Information showed in Table on immunocompetent or immunocompromised population aged less than 14 years, that health impacts reported to the population level are important.

The results presented in the previous paragraphs provide a first quantitative data on the risk of cryptosporidiosis in the population of Les Cayes. However, certain limits must be made, both on the analytic and the methodology level. Only five sampling campaigns have been conducted. This number is low and less than the number of recommended actions in order to obtain an estimate of the distribution of contamination (AFSSA, 2002). Since, estimations of the number of expected cases were made by using a strong assumption that all detected oocysts are viable, the result can have uncertainties in the estimations of the expected cases number. These conditions could lead to an underestimation or overestimation of the risk. Only an epidemiological survey in the population could allow to validate these first results.

4.4. Risk management of contaminated water from Les Cayes groundwater

In Haiti, the groundwater is contaminated by human and animal waste, therefore processing techniques must be developed for removal of *Cryptosporidium* of, at least, 3 log (99.9%). With such treatment, a concentration of 13 oocysts/100L of water from a source of water can be reduced to 1.3×10^{-2} oocysts/100L. This ability to reduce the contamination, that is the reduction of 3 log used, will provide drinking water, with an acceptable risk of infection for the general population. A significant benefit can be expected with a reduction of 2 log. For example, let's assume that the average annual dose ingested by a population of 5,200 inhabitants is 10^2 oocysts, using the risk estimation model described above, the number of cryptosporidiosis cases/year will be equal to 1,268 patients. With the installation of a small unit of water treatment with a reduction capacity of 2 log contamination, the number of cryptosporidiosis cases / year will be reduced to 20 patients.

The estimated impacts and the risks calculated in the framework of this thesis lead not only to highlight the relevance of prevention, but also of the need for medical care, the conduct of an epidemiological study and the implementation of microbiological monitoring of water resources feeding the population. It is particularly important to take these preventive and adapted measures in order to improve the health of people in the city of Les Cayes.

5. CONCLUSION

The approach developed to investigate the microbiological contamination by *Cryptosporidium* of the groundwater in the city of Les Cayes, and the risk assessment for consumers leads to a quantitative assessment of the risks of infection. It should be improved in relation to deeper soil analyzes enabling the study of the absorption kinetics and the hydrodynamic mechanisms of transfer of *Cryptosporidium* oocysts in groundwater. Moreover, the estimation of an average and its accuracy depending on the number of observations is a well-known statistical problem. The highly asymmetric and widely dispersed distribution of the contamination of a resource involves a large number of observations which are required for a good estimate of the average. It will then be necessary to carry out the verification of these first results and to couple them by measuring other indicators of fecal pollution of waters such as fecal coliforms and enterococci, other parasites (*Giardia* and helminthes) and the enteroviruses.

In the specific case of groundwater in the city of Les Cayes, it would be interesting to study, the efficiency of adsorption in zeolites oocysts. Indeed, zeolites are excellent ion exchangers their application in the treatment process of water contaminated with oocysts can probably reach a retention level higher than 91%.

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