PRESENCE OF CRYPTOSPORIDIUM OOCYSTS AND GIARDIA CYSTS IN THE SURFACE WATER AND GROUNDWATER IN THE CITY OF CAYES, HAITI.

PRESENCIA DE CRYPTOSPORIDIUM OOCYSTS Y GIARDIA CYSTS EN EL AGUA SUPERFICIAL Y EN EL AGUA SUBTERRÁNEA EN LA CIUDAD DE CAYOS (LES CAYES), HAITÍ.

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Abstract

The aim of this study was to determine the number of *Cryptosporidium sp* oocysts and *Giardia sp* cysts in the surface water and groundwater used by the population of the city of Cayes (Haiti). Samples of 3 to 200 litres of water were collected from 15 sites in and surroundings of the city (bathing water and household waste water, spring water, boreholes, water supply, domestic wells), and filtered using filter cartridges and stored at 4°C until examination. Oocysts and cysts were isolated using an immuno-magnetic method and counted under fluorescence microscopy after labelling with a monoclonal antibody. Eight specimens out of 15 (53%) contained *Cryptosporidium* oocysts and / or *Giardia* cysts. The number of *Cryptosporidium sp* oocysts detected varied from 5 to 100 (mean 29) / 100 L of water filtered and the number of *Giardia* cysts ranged from 5 to 960 (mean 277) / 100 L. Results suggest that surface water and ground water of the city of Cayes are contaminated by faecal pollution resulting in a potential risk for health of the population exposed.
Keywords: *Cryptosporidium sp*; *Giardia sp*; surface water; groundwater; drinking water; Haiti.

Résumé

Le but de cette étude était de déterminer le nombre d'oocystes de *Cryptosporidium sp* et de kystes de *Giardia sp* présents dans les eaux superficielles et souterraines utilisées par la population de la ville des Cayes (Haïti). Dans 15 sites répartis dans la ville, des volumes de 3 à 200 litres d'eau de différentes origines (eaux de baignade ou de travaux ménagers, eaux de source, de forage, de distribution, de puits domestiques) ont été recueillis, puis filtrés sur des cartouches de filtration conservées à 4°C jusqu'au traitement. Les oocystes et les kystes ont été isolés par une méthode immuno-magnétique et comptés au microscope à fluorescence après marquage par un anticorps monoclonal. Sur les 15 prélèvements d'eau analysés, 8 (53%) contenaient des oocystes de *Cryptosporidium* et/ou des kystes de *Giardia*. Le nombre d'oocystes de *Cryptosporidium sp* détecté variait de 5 à 100 avec une moyenne de 29 oocystes pour 100 litres d'eau filtrée ; pour les kystes de *Giardia* le nombre était compris entre 5 et 960 avec une moyenne de 277 kystes par 100 litres d'eau filtrée. Cette étude a montré que les eaux superficielles et souterraines de la ville des Cayes sont contaminées par une pollution d'origine fécale et constituent donc une source potentielle de risque biologique pour la santé de la population exposée.
Mots clés : Cryptosporidium sp ; Giardia sp ; eaux de surface ; eau souterraine ; eau de boisson ; Haïti.

Resumen

El objetivo de este estudio fue de determinar el número de ooquistes de *Cryptosporidium sp* y de quistes de *Giardia sp* presentes en las aguas superficiales y subterráneas utilizadas por la población de la ciudad de Los Cayes (Haití). Se colectaron en 15 sitios distribuidos en la ciudad, volúmenes de 3 a 200 litros de agua de diferentes orígenes (aguas dulce y de baño, aguas de manatial, de perforación, de consumo y de pozos domésticos), luego se trataron por medio de cartuchos de filtración que fueron conservados a 4°C hasta el procesamiento. Los ooquistes y los quistes han sido aislados por un método immuno-magnético y contados al microscopio a fluorescencia después de su marcación por un anticuerpo monoclonal. De las 15 deducciones analizadas de agua, 8 (53 %) contenían ooquistes de *Cryptosporidium* y/o quistes de *Giardia*. El número de ooquistes de *Cryptosporidium sp* detectado variaba de 5 a 100 con un promedio de 29 oocystes para cada 100 litros de agua filtrada; para los quistes de Giardia el número variaba entre 5 y 960 con un promedio

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de 277 quistes para cada 100 litros de agua filtrada. Este estudio mostró que las aguas superficiales y subterráneas de la ciudad de Los Cayes están contaminadas por una polución de origen fecal y constituyen a propósito una fuente potencial de riesgo biológico para la salud de la población expuesta a ella.

Palabras claves: Cryptosporidium sp; Giardia sp; aguas de superficie; agua subterránea; agua de bebida; Haití.

INTRODUCTION

Cryptosporidium sp and *Giardia sp* are widespread in the world, contaminating surrface water, river water, coastal water, groundwater and water supply systems. They are Protozoan parasite of prime concern due to their capacity to transmit endemic diseases (Anderson et al., 1998; US Geological Survey, 2006 a, b). They are eliminated with faeces in the form of oocysts and cysts (Benton, 1991; Craun and Calderon, 2006; Savioli, 2006), and are responsible of diarrhoea in infants and adults. The Center for Disease Control of Atlanta (United States) attributed 71% of the hydric diseases recorded in 1993 and 1994 in the United States to *Cryptosporidium parvum* and to *Giardia duodenalis* (Gostin et *al.* 2000).

Oocysts are highly resistant to different environmental constraints including standard chemical disinfection by chlorine and disinfection by free monochloramine, even after 18h of exposure (Korich et al., 1990; WHO, 2002; Standish-Lee and Loboschefsky, 2006). Under favourable conditions, they are capable of surviving for several months in the environment (Tamburrini et Pozio, 1999). In contrast, *Giardia* sp cysts are less resistant to disinfection by chlorine (Smith and Grimason, 2003). The accidental ingestion of oocysts and/or cysts in bathing water (Yorder et al*.,* 2004), water used for leisure purposes (Dziuban et al*.,* 2006), or consumption of contaminated drinking water (Mac Kenzie, 1994), expose the population to an infection risk The presence of oocysts and cysts in water is a significant risk factor for human health, especially for the most vulnerable groups (Craun et al., 2005; Coupé et al*.,* 2006; Raccurt, 2006).

In Haiti, *Cryptosporidium sp* is responsible for 17% of acute diarrhoeas observed in infants under 2 years of age (Pape et al., 1987) and 30% of chronic diarrhoeas in patients infected by HIV (Pape et al., 1983). In Port-au-Prince, *Cryptosporidium sp* oocysts were detected in surface water and in public water supplies (Brasseur et al., 2002). In the districts where water is contaminated, the risk of infection is estimated between $1x10^2$ and $5\ x10^2$ for immunocompetent and immunosuppressed population respectively. This calculated risk level varies from $1x10^2$ to $97x10^2$ according to the number of oocysts in the water consumed (Bras et al., 2007). These studies concerned exclusively the contamination of few aquatic ecosystems of the city of Port-au-Prince by *Cryptosporidium sp* and to date, no study on the contamination by *Giardia sp* in the country's lakes and ponds has been performed. The aim of this work was to carry out a preliminary study of the circulation of *Cryptosporidium sp* oocysts and *Giardia sp* cysts in the surface water and groundwater in Cayes, the third largest town of Haiti.

MATERIALS AND METHODS

Study site

The study site was the city of Cayes, the chief town of the South department with a population of 150,000 inhabitants. Likewise to all the towns of Haiti, Cayes has undergone considerable and rapid demographic growth over recent years (1950: 11,600 inhabitants; 1971: 22,000 inhabitants; 2007: 150,000 inhabitants). Cayes is located on the coast facing the Caribbean Sea, on a well-watered coastal plain (rainfall > 2,000 mm/yr), at 18°34'00'' Northern Latitude and 72°21'00" Longitude. The average temperature varies from 24°C to 28°C. There are two successive rainy seasons: from April to May and August to October.

Ravine du Sud, main river in the town, has flow rate in average of 4.96 m^3/s and a low water flow rate of 1.31 m^3/s. The watershed is covered with lagoon-lacustrine and marine sediments, respectively from the lower and middle Miocene. It is divided between 3 distinct types of groundwater: alluvial aquifers in free ground water, karstic aquifers, and cracked and compartmented carbonaceous aquifers, giving rise to varied resurgences and flows (UNDP, 1991).

Sampling points

Water samples were taken in September, November and December 2007, i.e. at the end of the main rainy season and at the beginning of the main dry season. The sampling points were chosen according to the water supply points of the population. . The water samples were collected from the 15 sites in the conditions defined by standard AFNOR-NFT 90-455 of July 2001. Water samples were filtered using filter cartridges (Envirocheck®, Pall Gelman, Saint Germain en Laye, France). The quantities filtered varied from 3 to 200 litres depending on the turbidity level. Cartridges were then stored at 4°C until examination. A GPS was used to record the geographical coordinates of the sampling points selected.

Analysis of the samples

Oocysts and cysts purification

Cryptosporidium oocysts and Giardia cysts were separated using immunomagnetic beads coated with an anti-*Cryptosporidium* monoclonal antibody and anti-*Giardia* cyst (Dynabeads®, Dynal, Oslo, Norway) according to manufacturer's instructions. Briefly, 300 to 600 µl of centrifuge pellet was introduced in Leighton tubes and adjusted to 10 ml with deionised water. Anti-*Cryptosporidium* and anti-*Giardia* paramagnetic beads were added and incubated on a rotating mixer at room temperature for 1h. Then, the paramagnetic bead complexes were captured using a magnetic concentrator. Oocysts and / or cysts were dissociated from beads using an acid solution (0.1 N HCl). The acid suspension of ocysts and / or cysts obtained was neutralized by adding 5 µL of 1 N NaOH. Detection and counting of oocyst and cyst were performed using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (MAb) directed against a *Cryptosporidium* and a *Giardia* wall antigen . (FITC-Cow MAb, Monofluokit *Cryptosporidium*® and *Giardia*, Bio Rad, Marnes la Coquette, France). A positive control slide was prepared by drying a 200 µl aqueous suspension containing approximately 10^4 *C. parvum* oocysts from feces of calves experimentally infected with an isolate maintained by M. Naciri, (Laboratoire de Pathologie aviaire, Institut National de la Recherche Agronomique, Nouzilly, France) purified using density separation (1) Results were expressed as number of oocysts or cyst per 100L of water filtered.

RESULTS

Cryptosporidium sp oocysts and / or *Giardia sp* cysts were found in 8/15 (53%) samples analysed (Table 1). Samples from the 6 sites contaminated by *Cryptosporidium sp* contained 5 to 100 oocysts for 100 L of water collected, i.e. an average of 29. The samples of the 4 sites contaminated by *Giardia sp* contained 5 to 960 cysts, i.e. an average of 277 cysts for 100 L (table 2). Table 3 shows the levels and types of contamination and the sources of water. The geographical distribution of the water points tested in the city of Cayes and the average concentration in oocysts and cysts are shown in figure 1.

DISCUSSION

During this study, the water from wells or distributed by the local company supplier was clear and large quantities of water were filtered (120 to 200 L). In contrast, for both surface water and water from certain wells, the quantities of water filtered were limited because of a heavy amount of suspended organic particle rapidly clogged the filters. When the turbidity was highest such as water collected in the estuary of the Islet river (number 2) and in the Ravine du Sud (number 1), the quantities filtered were too low (respectively 3.1 and 7.5 L) for assessment of absence of parasite and a significant relationship with a faecal contamination.

The results were obtained according to the standard method based on filtration, elution, and the concentration of oocysts and cysts by immunomagnetic separation which is considered as an efficient tool for detecting and identifying oocysts and cysts in environmental samples (USEPA, 1999 a and b; Smith et al, 2006). In addition, this method reduces the number of false positives and gives better microscopic results, but it requires a large quantity of water (Connell et al., 2000).

Results obtained for *Cryptosporidium sp* and *Giardia sp* confirm that the population of Cayes city is exposed to parasitic contamination transmitted by drinking water. , Although it has been reported that a median infectious dose of 132 oocysts in healthy adult volunteers, provoked a human infection in 50% of cases (Dupont et al., 1995), a mathematical model based on the data from the Milwaukee outbreak suggested that some individuals developed a cryptosporidiosis after ingestion of only one oocyst (Haas et Rose, 1994). Rose, in 1990 estimated that an annual risk 1/10,000 *Giardia* infection would result from an exposure to an annual geometric mean of 0.0007 cysts per 100 L of water. Depending on the level of contamination in the raw water supply, utilities will have to apply treatment to achieve a geometric mean of less than 0.0007 cyst per 100 litres in treated water.

Giardiasis is a common cause of diarrhoea in man, and a chronic infection in infants, resulting in poor food absorption and in interrupted growth (Thompson and Monis, 2004). Cryptosporidiosis is particularly serious in under-nourished persons and immunosuppressed patients, especially those infected by human immunodeficiency virus (HIV). In immunocompetent subjects, the symptoms are relatively benign (Tzipori et al, 1983; Jokipii and Jokipii, 1986), while in AIDS patients it is one of important cause of morbidity and mortality (Pape and Johnson, 1993). In infants, it causes prolonged diarrhoea, malnutrition, and possible delayed psychomotor development (Agnew et al, 1998; Sanchez-Vega, 2006). According to PSI-UNFPA (2005), 12% of boys and 9% of girls aged from 19 to 24 years old are infected by HIV in the region of Cayes.

In the samples studied, *Cryptosporidium sp* oocysts were found more often (6/15) although *Giardia sp* cysts were more abundant on average. The largest numbers of oocysts and cysts were identified in sample 4 (Pont de la rivière l'Islet). This point of surface water, partly covered by aquatic plants (water hyacinth = *Eichhornia Crassipes)* is used as a watering place for free-roaming livestock (pigs, horses and cattle) (Photo 1). These water resources are used for bathing and washing clothes by the population only a few metres from its point of discharge into the sea. For the other surface waters used by the population either for domestic requirements (dish-washing), or for pleasure (bathing), this study shows that the water in the lagoon of Gelée beach, very frequently used for bathing and leisure, was heavily contaminated at the time of the survey, during the rainy season. Indeed, filtration highlighted 24 *Cryptosporidium sp* oocysts and 139 *Giardia* cysts. This contamination of faecal origin increases the risk of outcome of parasitic disease and for health of vulnerable individuals (Coupe et al., 2006) on a beach attracting many inhabitants from Cayes and other parts of Haiti during holidays.

Among the different contaminated sites identified, the number of oocysts obtained for site 5 raises a serious concern since located within the perimeter of a pumping station of the public water production and supply network serving the town's population. The contamination of the water produced by this service has already been reported in the technical literature on the microbiological quality of the water distributed in Haiti and 110 faecal coliforms per 100 mL water were detected in the water of this network (sub-committee responsible for drinking water and the removal of human waste, 1991). Detecting *Cryptosporidium sp* in these waters is especially worrying for vulnerable populations (elderly, children, and immunosuppressed) and can contribute to increase the mortality rate in this city due to the lack of an effective treatment. (MacKenzie et al., 1994; Rose et al., 1991; Wright et Collins, 1997; WHO, 2002).

The presence of *Cryptosporidium sp* in the public water supply system can be explained by several factors. (i) There is no water quality control performed for public water supplies in Haiti (Ministry of Public Health and WHO, 1998). (ii) Chlorination remains the only method of treating raw water intended for human consumption (Emmanuel and Lindskog, 2002). It has been shown that disinfection by chlorination is ineffective in inactivating *Cryptosporidium sp* oocysts (Korick et al., 1990; Lorenzo-Lorenzo et al., 1993). *Giardia* cysts are susceptible to inactivation with chlorine but at extended contact times (Rice and Hoff, 1981). This may explain the absence of *Giardia* cysts in sample 5. Inactivation of *Cryptosporidium sp* oocysts is simply impracticable using chlorine; filtration may be the most appropriate barrier (Korick et al., 1990; Gyüré et al., 1997). (iii) The city of Cayes is characterised by the absence of basic services, such as the collection and treatment of wastewater, the collection of solid wastes and the removal of excreta. (iv) The presence of latrines and septic tanks in the hydraulic perimeter of the wells supplying the town with drinking water. This situation can contribute towards contaminating the water resources available in the groundwater by human faeces. (v) One of the major characteristics of the geology and hydrogeology of Cayes is the presence of a karstic aquifer (UNDP, 1991). The main characteristic of karstic aquifers is the existence of irregular networks of pores, cracks, fractures and conduits of various forms and dimensions. The considerable physical and geometric heterogeneity of this type of structure gives rise to complex hydraulic conditions varying in space and time. Following a rainfall, the replenishment of the groundwater is both rapid and turbulent, with the drainage of high volumes of non-filtered water in large conduits (Denić-Jukić and Jukić, 2003).

These results highlight significant contamination of the surface water and groundwater resources of Cayes by Cryptosporidium sp and Giardia sp, and underline the existence of a biological danger for the population exposed. The presence of Giardia sp and Cryptosporidium sp in water resources requires further study. Contamination levels may fluctuate significantly, as they are influenced by a variety of poorly defined factors, such as the climate, e.g., flooding, agricultural practices and free-roaming livestock (cattle, poultry), on-site sanitation and pit latrines in karstic aquifers, and the sporadic nature of the deposition of animal faeces containing cysts and oocysts. Unfortunately, there are no reliable methods of determining the viability of the individual cysts and oocysts observed in environmental samples. Hence additional research to develop reliable methods of determination and studies should also be performed to evaluate the virulence of environmental cysts and oocysts. It is now necessary to validate these initial data by characterising the parasites found and apply molecular genotyping techniques to Cryptosporidium sp oocysts and Giardia sp cysts at the sites studied and at new ones to be chosen in Cayes. It would also be of interest to combine these parasitological examinations with the bacteriological characterisation of the water points, for example, by seeking Escherichia coli, and the determination of several physicochemical parameters such as pH, electrical conductivity, total dissolved solids and turbidity.

Table 1: Distribution of 15 sites studied for the presence or absence of*Cryptosporidium sp* oocysts and/or *Giardia sp* cysts

Water filtration	n	%
Absence of parasitic organisms	7	47%
Presence of Cryptosporidium sp oocysts	4	27%
Presence of giardia Giardia sp cysts	2	13%
Presence Cryptosporidium sp oocysts and Giardia sp cysts	2	13%
Total	15	100%

Table 2: Number of *Cryptosporidium sp* oocysts and *Giardia sp* cysts obtained in the samples studied.

No	Number of litres of water filtered	Number of oocysts /100 L	Number of cysts /100L	Type of water
1	7.5	0	0	Surface water
2	3.1	0	0	Surface water
3	120	0	0	Well water
4	20	0	960	Surface water
5	200	9	0	Water supply network
6	13	24	139	Surface water
7	200	0	0	Well water
8	30	0	0	Domestic well
9	63	100	0	Domestic well
10	132	14	0	Surface water
11	15	0	0	Surface water
12	12	0	0	Surface water
13	200	23	0	Well water
14	200	5	5	Well water
15	200	0	5	Well water

Table 3: Levels and types of contamination of the water studied in the city of Cayes

Type of water		Absence of parasitic organisms	Crypto oocysts	Giardia cysts	Crypto + Giardia	% of contaminated water
Surface water	River water (bathing)	2		1		33%
	Lagoon water (bathing)				1	100%
Groundwater	Spring water	2	1			33%
	Well water	1	1			50%
	Borehole water	2	1	1	1	60%
	Water supply		1			100%
	Total number	7	4	2	2	53%

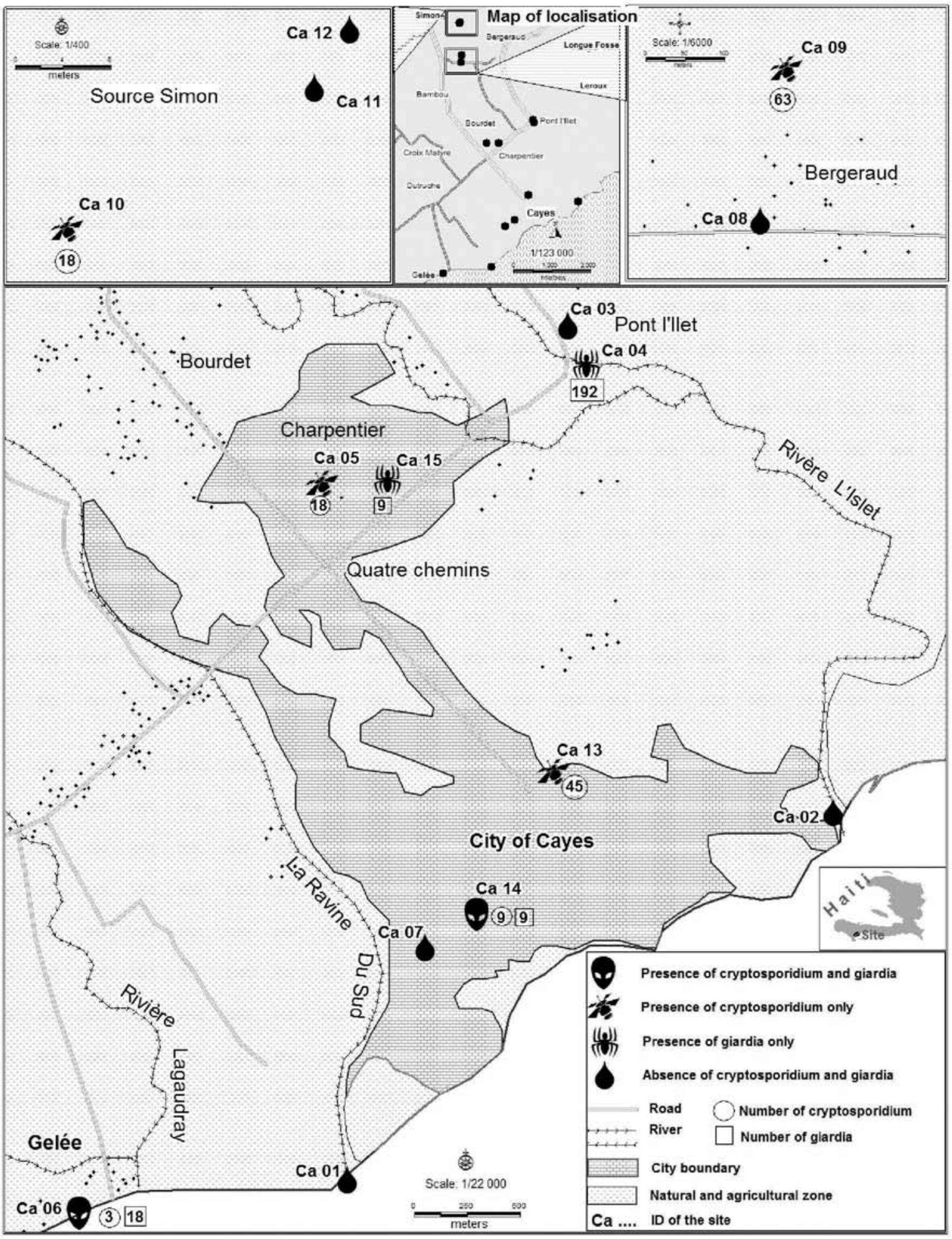

Figure 1. Graphic representation of the contamination of water sites studied in the region of Cayes in southern Haiti (number of *Cryptosporidium* oocysts and *Giardia* cysts for 100 litres of filtered water).

Photograph 1: Bridge over The Islet River at the eastern entry of Cayes (no. 4): water loaded with organic and sediment material clogged by aquatic plants and frequented by domestic animals.

CONCLUSION

This study highlighted that the surface water and groundwater of the town of Cayes are considerably contaminated by C*ryptosporidium sp* and *Giardia sp*, two enteric Protozoa responsible for hydric diseases in man, with potentially serious complications in vulnerable subjects. Their potentially high prevalence in source water used to supply the population, their resistance to conventional water treatment, the lack of effective treatment and the absence of adequate techniques to detect the presence of infectious oocysts and cysts, requires the consistent and effective removal of these parasites from the water supply. There is a clear need for utilities to perform evaluations of raw water parasite levels in order to determine the appropriate level of treatment.

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