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Prospective study of acute health effects in relation to exposure to cyanobacteria

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HIGHLIGHTS

- The relationship between exposure to cyanobacteria and health symptoms was examined.
- Limited water contact with cyanobacteria was linked to gastrointestinal symptoms.
- Drinking water contaminated by cyanobacteria was associated with health symptoms.
- The public should be informed of symptoms associated with exposure to cyanobacteria.
- A management plan is needed for plants treating cyanobacteria-contaminated water.

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ABSTRACT

We conducted a study to investigate the relationship between exposure to cyanobacteria and microcystins and the incidence of symptoms in humans living in close proximity to lakes affected by cyanobacteria. The design was a prospective study of residents living around three lakes (Canada), one of which has a water treatment plant supplying potable water to local residents. Participants had to keep a daily journal of symptoms and record contact (full or limited) with the water body. Samples were collected to document cyanobacteria and microcystin concentrations. Symptoms potentially associated with cyanobacteria (gastrointestinal: 2 indices (GI1: diarrhea or abdominal pain or nausea or vomiting; GI2: diarrhea or vomiting or [nausea and fever] or [abdominal cramps and fever]); upper and lower respiratory tract; eye; ear; skin; muscle pain; headaches; mouth ulcers) were examined in relation with exposure to cyanobacteria and microcystin by using Poisson regression. Only gastrointestinal symptoms were associated with recreational contact. Globally, there was a significant increase in adjusted relative risk (RR) with higher cyanobacterial cell counts for GI2 (<20,000 cells/mL: RR = 1.52, 95% CI = 0.65–3.51; 20,000–100,000 cells/mL: RR = 2.71, 95% CI = 1.02–7.16; >100,000 cells/mL: RR = 3.28, 95% CI = 1.69–6.37, p-trend = 0.001). In participants who received their drinking water supply from a plant whose source was contaminated by cyanobacteria, an increase in muscle pain (RR = 5.16; 95% CI = 2.93–9.07) and gastrointestinal (GI1: RR = 3.87; 95% CI = 1.62–9.21; GI2: RR = 2.84; 95% CI = 0.82–9.79), skin (RR = 2.65; 95% CI = 1.09–6.44) and ear symptoms (RR = 6.10; 95% CI = 2.48–15.03) was observed. The population should be made aware of the risks of gastrointestinal symptoms associated with contact (full or limited) with cyanobacteria. A risk management plan is needed for water treatment plants that draw their water from a source contaminated with cyanobacteria.

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1. Introduction

Cyanobacteria occur naturally in freshwater and marine ecosystems in all climates (AFSSA-AFSSET, 2006). These microorganisms are of interest to researchers and public health authorities because of the toxicity of the cyanotoxins they produce.

Cyanotoxins are a large group of toxins that produce a variety of negative health impacts. Some cyanotoxins are hepatotoxins, including nodularins and microcystins, of which there are at least 80 variants (Humpage, 2008). Microcystins are the most frequently documented toxins in blooms (WHO, 1999) in human and animal poisonings (AFSSA-AFSSET, 2006). A cytotoxin, cylindrospermopsin, has also been documented, mainly in Australia but also in Europe, Asia, South America and North America (Falconer and Humpage, 2005; Hamilton et al., 2005). Some cyanotoxins are neurotoxins (AFSSA-AFSSET, 2006), including anatoxin, saxitoxin and its derivatives and β-N-methylamino-L-alanine (BMAA), which is suspected of being associated with neurodegenerative diseases (Pablo et al., 2009). Lastly, endotoxins, structural components of cyanobacterial cell walls, are suspected of having irritative or allergenic effects (AFSSA-AFSSET, 2006).

In 1996, an unfortunate incident occurred in Caruaru, Brazil that highlighted the toxic potential of cyanotoxins. At a hemodialysis unit, one hundred people developed liver failure after undergoing treatment with water contaminated by microcystins (Carmichael et al., 2001).

Acute effects have been associated with drinking water, and several case series have been reported following exceptional situations, such as the use of copper sulfate to treat blooms in drinking water reservoirs or the inappropriate treatment of massive blooms. The most common health problems were gastrointestinal or hepatic symptoms (Hawkins et al., 1985; Lippy and Erb, 1976; Teixeira Mda et al., 1993; Veldee, 1931). A case–control study also showed an increase in gastrointestinal and dermatological symptoms in people whose drinking water supply came from a river which was contaminated with an extensive bloom and was chlorinated but otherwise not treated (El Saadi et al., 1995). While the World Health Organization (WHO) and Health Canada have established guidelines for total microcystin-LR in drinking water of 1 μg/L (WHO, 2008) and 1.5 μg/L (HC, 2013) respectively, there is a need for data from epidemiological studies on the health effects of exposure to cyanobacteria to clarify the risk (Falconer and Humpage, 2005).

Case series have also been published regarding recreational exposure, with the most common symptoms being gastrointestinal, cutaneous and respiratory (Dillenberg and Dehnel, 1960; Turner et al., 1990; Williamson and Corbett, 1993). As with drinking water, few epidemiological studies with comprehensive study designs have been conducted. Two prospective studies conducted on visitors exposed to cyanobacteria at different beaches showed associations with health effects (Pilotto et al., 1997; Stewart et al., 2006a). For recreational water, the WHO has estimated that there is a low probability of adverse health effects below 20,000 cells/mL, while there is a moderate risk of effects, in particular acute gastrointestinal or skin problems, at concentrations over 100,000 cells/mL because at this level, 20 μg/L of microcystins may be present (WHO, 1999). Similarly, Health Canada has established guidelines of 100,000 cells/mL and 20 μg/L for total cyanobacteria and total microcystins, respectively (HC, 2012).

To further substantiate the limited data on the acute human effects following drinking water and recreational water exposure to waterborne cyanobacteria, we conducted a prospective study over an eight-week period on people living on the shores of three lakes in the province of Quebec (Canada). Our objective was to investigate the relationship between exposure to cyanobacteria and microcystins (the most common cyanotoxins) and the incidence of symptoms in human populations living in close proximity to the lakes affected by cyanobacteria.

2. Material and methods

On the basis of their history of cyanobacterial presence (from a list prepared by the Quebec Ministry of Sustainable Development, Environment and Parks), the presence of an active residents' association willing to collaborate in the project and proximity to the research team, three lakes were selected: Lake William (LW), Lake Roxton (LR) and Lake Champlain's Missisquoi Bay (MB). At the latter site, a drinking water treatment plant supplied a sector of MB residents. The inclusion factors for study participants were: five years of age or over, access to the lake and residing in the targeted residence for more than two weeks during the study period. No more than three participants per family were accepted. Approximately 400 addresses were randomly selected for each lake. Data collection took place from June 27 to August 21, 2009. The final toxin analyses were completed in December 2011. The protocol was approved by the research ethics committee of the Centre Hospitalier Universitaire de Québec and participants signed an informed consent form.

Two tools were used for data collection. First, a *family questionnaire* was used to document different individual variables (sociodemographic characteristics, medical history, symptoms in the two weeks prior to data collection, medication, occupation, travel in the last month) and information about the household (drinking water supply [treatment plant drawing its water from MB, water at risk of fecal contamination (surface well, water taken directly from the lake), others], presence of pets at home) and a *daily journal of symptoms* to collect data from participants on symptoms (eye, ear, respiratory, gastrointestinal, skin, muscle pain, headaches, mouth ulcers) potentially associated with cyanobacteria (Pilotto et al., 1997; Stewart et al., 2006a) including medical consultations or hospitalizations. At the same time, participants recorded full contact (swimming, waterskiing, windsurfing, use of watercraft involving launching, accidental falls) and limited contact (fishing, use of watercraft not involving launching) with lake water, the duration (less than 1 h, 1 h to less than 3 h, 3 h and more) and location of contact as well as head immersion and ingestion of water or not during water contact. Lastly, swimming in other lakes or rivers, in swimming pools and consumption of fish from the lake were also recorded.

Daily surface water samples (duplicates at a water depth of 0.3 m and duplicates from 1.2 m water depth (MENVIQ, 1986) comprised one composite sample) were collected at five littoral sampling stations at LW and MB, and at four stations at LR. Limnetic samples were also collected (3 surface water samples to form a composite sample) twice a week at one station at LR and at two stations at LW and MB. Once a week, samples (littoral: 1 where water depth was 0.3 m and 1 where water depth was 1.2 m to form a composite sample; limnetic: 1 sample) were also collected at a depth of 15 cm to assess for the presence of *Escherichia coli* (*E. coli*) at the same stations. For days without sampling and other missing data (technical problems), values were interpolated for each day based on the chronologically closest values measured. For cyanobacteria, microcystins and *E. coli*, 3%, 7% and 87%, of the littoral samples and 86%, 85% and 88% of the limnetic samples were interpolated, respectively.

Cyanobacterial cells were counted and identified as previously described (Rolland et al., 2005). Dissolved microcystin was measured in filtered water samples, which were passed through glass fiber filters of 0.7 μm pores (Whatman GF/F) and kept frozen. Microcystin particulates were captured on the same filter using volumes ranging from 20 to 500 mL, depending on the density of planktonic particles. Particulate toxins were extracted by sonication of frozen filters in distilled water, followed by clarification by centrifugation at 13,000 *g* for 10 min. The final extracts were diluted 20 to 40 times in order to avoid interferences present in the raw water. After filtration, dissolved and particulate microcystins were determined by ELISA (Abraxis 96-well microtiter plates PN 520011, Abraxis, Warminster, PA). This test is sensitive to the functional moiety of these hepatotoxins, the ADDA group, but the