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Short Communication

Exposure to cyanobacteria: acute health effects associated with endotoxins

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We recently conducted a prospective epidemiological study to document the acute health effects in relation to exposure to cyanobacteria in three lakes located in Quebec (Canada).[1] Only gastrointestinal symptoms were found to be associated with contact with the lakes, and there was a significant relationship with counts of cyanobacteria for which participants were exposed to greater concentrations of cyanobacteria.[1]

Some of the symptoms related to endotoxin exposure in humans are gastrointestinal.[2] We measured the endotoxin concentrations in water samples taken during the aforementioned study, with the goal of examining the relationship between endotoxin exposure and the incidence of symptoms in the study participants.

The population choices for the study and the data collection methodology have been described elsewhere.[1] Briefly, three lakes were selected on the basis of their history and frequency of cyanobacterial blooms: Lake William (LW), Lake Roxton (LR) and Lake Champlain's Missisquoi Bay (MB). At the latter site, a drinking water treatment plant supplies some MB residents. Data were collected from June 27 to August 21, 2009. The protocol was approved by the research ethics committee of the Centre Hospitalier Universitaire de Québec and participants signed an informed consent form.

A daily journal of symptoms was used to collect data from participants on symptoms (eye, ear, respiratory, gastrointestinal, skin, muscle pain, headaches, mouth ulcers) potentially associated with cyanobacteria.[3] By using a list of activities, participants also recorded full contact (swimming, water-skiing, wadeboard, windsurfing, kite surfing, diving, use of

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watercraft involving launching, accidental falls, relaxing on a floating chair or mattress, dock repair with contact with water) and limited contact (fishing, use of watercraft (kayak, pontoon boat, canoe, dinghy, sailboat) not involving launching) with lake water, the duration and location of contact, head immersion and water ingestion.

Daily surface water samples were collected at littoral stations (five: LW-MB; four: LR). Limnetic samples were also collected twice a week (one station: LR; two stations: LW-MB).[1] For days without sample and other missing data (technical problems), values were interpolated for each day based on the chronologically closest values measured. For endotoxins, 23% of the samples were interpolated (limnetic: 81%; littoral: 2%).

We used frozen filters (−80 °C) from these samples to document the presence of endotoxins. Endotoxins were determined by a *Limulus* amoebocyte lysate (LAL) test (Charles River Laboratories, Boston, MA).

For respiratory symptoms, two indices were created: upper respiratory tract (runny nose, sore throat) and lower respiratory tract (cough, wheezing). Two indices were also created for highly credible gastrointestinal symptoms: GI1 (diarrhoea [3 loose movements/day], abdominal pain, nausea or vomiting) and an index for more severe symptoms, GI2 (diarrhoea, vomiting, nausea and fever, or abdominal cramps and fever).[4] A six-day symptom-free interval was used to distinguish between episodes. We verified for the presence of contact with the lake on the same day and in the three days prior to the episode for muscle pain and gastrointestinal symptoms, and on the same day and the day prior to the episode for other symptoms.[1]

Endotoxin and cyanobacterial counts were slightly correlated (polychoric correlation coefficient = 0.57). Therefore, the analysis of the relationship between health effects and endotoxin concentrations was considered to be pertinent. Poisson regression was used to model the incidence of the different symptoms in relation to contact with water endotoxins (unexposed and three strata of exposure in tertiles; unexposed and two strata of exposure, the two first tertiles versus the third tertile) taking into account age and sex and potential confounders (family income, education, occupation, medical history, drugs, contact with children younger than 5 years old, contact with pets, foreign travel, source of drinking water of the dwelling, domestic treatment of drinking water, bathing in another lake, bathing in a swimming pool) found to be associated with symptoms in bivariate analysis ($P \leq 0.1$). When appropriate, trend tests were calculated using orthogonal contrasts. It was not possible to examine symptoms by type of contact (full or limited) in relation to concentrations in three strata (tertiles) due to the small sample size.

A total of 267 families and 466 subjects (245 women, 221 men) were included in the study (LW, 95 families, 155 participants; LR, 83 families, 150 participants; MB, 89 families, 161 participants; age distribution: 0–20 years: 11%; 21–40 years: 12%; 41–60 years: 39%; >60 years: 38%). For the drinking water source of the dwellings, we identified households with a source at risk of cyanobacterial or faecal contamination. Twenty families were supplied by a water treatment plant collecting water in MB in an area contaminated by cyanobacteria and 18 used a source at risk of faecal contamination (surface well, 15; directly in the lake, 3). Others were considered not at risk (artesian wells, municipal and private water plants with an adequate treatment and with a source not at risk of contamination by cyanobacteria).

Median endotoxin concentrations were lower for limnetic stations than for littoral stations. For LR, endotoxin concentrations were generally higher (littoral: median = 57 endotoxin units (EU)/mL, range: 13–437 EU/mL; limnetic: median = 22 EU/mL, range: 12–42 EU/mL) than in LW (littoral: median = 30 EU/mL, range: 0.72–109 EU/mL; limnetic: median = 16 EU/mL, range: 9–42 EU/mL). However, they were particularly high in MB for littoral stations (littoral: median = 375 EU/mL, range: 16–7683 EU/mL; limnetic: median = 25 EU/mL, range: 7–326 EU/mL). As a whole, there was much more variation in littoral stations than in limnetic stations. Considering that most of the interpolated results were for limnetic stations, these results are reassuring concerning the precision of the interpolated data.

None of the symptoms other than gastrointestinal were associated with exposure to endotoxins and there was no medical consultation following these episodes. Unfortunately, due to the limited number of episodes, it was not possible to verify the relation between GI2 and the concentrations of endotoxin by type of contacts even when stratified in two strata. The multivariate model for GI1 adjusted for age, sex, the residence's water source and the gastrointestinal symptoms reported in the two weeks prior to data collection, showed an increase in the adjusted RRs in relation to the endotoxin concentration for the two types of contact (full contact: two first tertiles (≤48 EU/ml), RR = 0.47 (95% CI = 0.19–1.16); third tertile (>48 EU/ml), RR = 2.12 (95% CI = 0.97–4.62); limited contact: two first tertiles (≤48 EU/ml), RR = 2.12 (95%CI = 1.17–3.85); third tertile (>48 EU/ml), RR = 3.43 (95%CI = 1.85–6.34)). There was no relationship with the duration of contact, head immersion and water ingestion.

Table 1 presents the relationship between gastrointestinal symptoms and exposure to endotoxins distributed in tertiles. For GI1 and GI2, there was a significant trend of increased RRs in relation to the concentration of endotoxins. We verified the models by including the degree of exposure to cyanobacteria during the contacts and there was almost no effect on the relationship with endotoxins for GI1 (first tertile, RR = 1.32 (95%CI = 0.67–2.59); second tertile, RR = 1.30 (95% CI = 0.62–2.75); third tertile, RR = 3.00 (95%CI = 1.69–5.32)) and for GI2 (first tertile, RR = 0.99 (95%CI = 0.34–2.92); second tertile, RR = 2.00 (95%CI = 0.78–5.09); third tertile, RR = 3.22 (95%CI = 1.64–6.32)). As previously documented, the RRs were high for families whose drinking water supply came from MB, especially for GI1, and for families living in houses with water sources at risk of faecal contamination.[1] Moreover, there was no relationship between gastrointestinal effects and exposure to *E coli*.[1]

The LAL test, the most widely used method for endotoxin detection,[5] was used to determine endotoxin concentrations. However, after filtration, the filters of our samples were kept frozen at −80 °C for many months. O'Toole et al. showed a 44% mean decline in the concentration of endotoxin in water samples stored at −80 °C for 4 weeks compared to samples kept at 4 °C for 24 h.[6] Therefore, the concentrations documented could be underestimated and we think that they should be interpreted on an ordinal basis.