Microbial sources tracking in recreational water in a leisure center.

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Introduction

Recreational activities on the Champs-sur-Marne leisure area are regularly affected by water contamination issues associated with pathogens of fecal origin. The objective was to identify the origin of these microbes to provide technical development and management solutions favorable to the improvement of the water quality and compatible with bathing activity.

A part of this project was to test the hypothesis of bacteriological contamination by aquatic birds massively occupying the leisure center (fig.1) using a microbial source tracking technique. This approach relies on the quantification by real-time PCR (qPCR) in water, sand, and sediment of bacterial specific markers of the digestive tract of different animal species (geese, seagulls, dogs) and humans.

Figure 1: Scarecrow (Eagle) to scare off birds.

Materials and methods

Sampling strategy

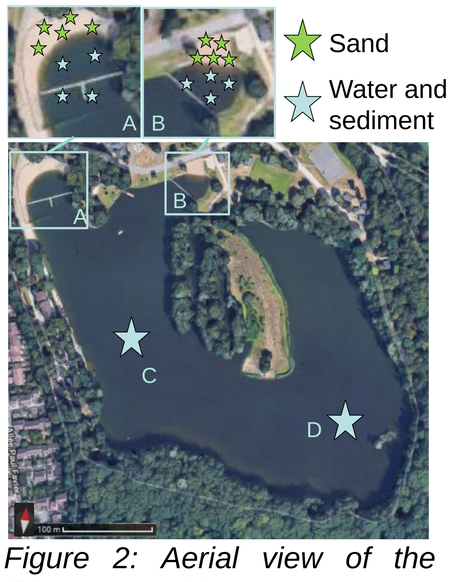

Figure 2: Aerial view of the Champs-sur-Marne leisure lake.

Two sampling campaigns took place in late June and late August 2015 at the bathing areas (A and B) and in the lake (C and D) (fig.2) in 4 replicates:

- 2L water for qPCR analysis
- 10L water for virus analysis
- 2x50mL tubes of sediment

In duplicates :

- 2 tubes 50mL of mixed sand from quadrat 50x50x1cm on beach

Analysis

Samples → DNA extraction → qPCR

Target	Organism	Gene	Primers 5'-3'	Probe
Dog	Bacteroidales	16S rRNA [1]	GGA GCG CAG ACG GGT TTT CAATCGGAGTTCTTCGTGATATCTA	FAM-TGG TGT AGC GGT GAA A-MGB
Goose	Bacteroidales	16S rRNA [2]	GTAGGCCGTGTTTTAAGTCAGC AGTTCCGCCTGCCTTGTCTA	FAM - CCG TGC CGT TAT ACT GAG ACA CTT GAG - BHQ1
Seagull	*Cattelicoccus marimammalium*	16S rRNA [3]	CTTGCATCGACCTAAAGTTTTGAG GGTTCTCTGTATTATGCGGTATTAGCA	FAM-ACA CGT GGG TAA CCT GCC CAT CAG A - BHQ1
Birds	*Campilobacter lari*	PepT (pepidase T)[4]	TTAGATTGTTGTGAAATAGGCGAGTT TGAGCTGATTTGCCTATAAATTCG	FAM-TGAAAATTGGAAdCGdCAGGTG-BHQ1
Human	*Campilobacter jejuni*	HipO (Hipurase O) [4]	TGCACCAGTGACTATGAATAACGA TCCAAAATCCTCACTTGCCATT	FAM-TTGCAACCTCACTAGCAAAATCCACAGCT-BHQ1
Human	Enterovirus, adenovirus, influenza virus A , B	5' UTR Enterovirus Hexon Adenovirus [5] Segment M and NS1 [6]	[5] and [6]	[5] and [6]

For each bacterial target, a plasmid vector was built for the standard curve. Additionally, another plasmid was used as an internal inhibition control. For virus analysis, a control DNA was added to the samples.

Results

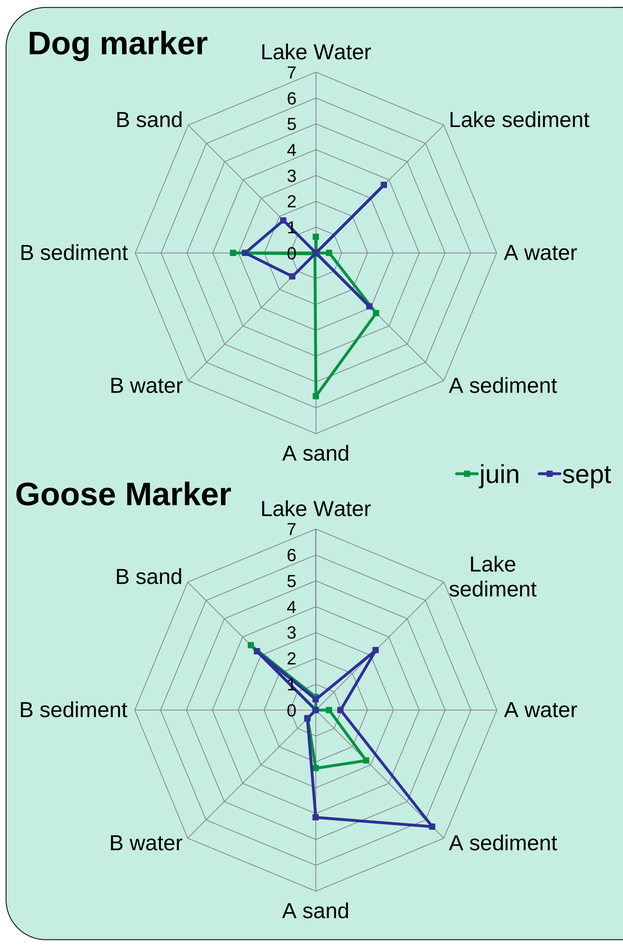

Figure 3: number of genome copies of dog and goose markers (average Log+1). Lake values are averages of C and D values.

Bacterial sources:

- Gull marker was detected only in the water from the large bathing area (A) in September, and in low quantity (less than 3 genome copies per ml).
- *C. jejuni* was detected in just one sample of lake water with a quantity of 0.2 genome copies per ml.
- Dog bacterial marker was detected in most samples with a large quantity range (0.4 to 1.6 x 10^7 genome copies per ml or g). Dog marker is present in both sediment and sand of the bathing areas, and less in the water. The patterns differ between June (B is more contaminated) and September (A is more contaminated).
- Goose bacterial marker was detected in all types of samples ranging from 1 to 9.4 x 10^6 genome copies per ml or g. Goose marker was predominant in the sand and the sediment of the large bathing area (A). The pollution was higher in September than in June.
- Dog and goose markers were more present in the lake sediment than the lake water in September.

Virus:

- Influenza virus A and B were not detected (qPCR was not inhibited).
- Human enterovirus and adenovirus were not detected in 10L, but some samples were inconclusive (qPCR amplification inhibition).

Conclusions

No contamination by human enteric viruses was found. This suggests that the contamination was not from human origin.

The detection of animal markers in all matrices before and after the swimming season indicates that dogs and geese contribute to the most to the fecal contamination of the water, sand and sediment. The contamination could be originated from two watchdogs roaming around the lake during closing hours, and several geese settled around the large bathing area or on the dock. The fences around the beaches do not stop geese and dogs. Thus, fecal contamination may occur by direct defecation of the birds in the water or by runoff during rain events from the grass, sand and docks.

These results are reinforced with a microbial community analysis where sequencing patterns from the lake samples (water and sediment) and different fecal sources were compared (animal feces, urban waste water, and urban runoff).

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