Prevalence and Occurrence of Zoonotic Bacterial Pathogens in Surface Waters Determined by Quantitative PCR

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Summary

The prevalence and concentrations of *Campylobacter jejuni*, *Salmonella* spp. and enterohaemorrhagic *E. coli* (EHEC) were investigated in surface waters in Brisbane, Australia using quantitative PCR (qPCR). Water samples were collected from Brisbane City Botanic Gardens (CBG) Pond, and two urban tidal creeks. Of the 32 water samples collected, 8 (25%), 1 (3%), 9 (28%), 14 (44%), and 15 (47%) were positive for *C. jejuni mapA*, *Salmonella invA*, EHEC O157 LPS, EHEC VT1, and EHEC VT2 genes, respectively. The high prevalence, and concentrations of potential zoonotic pathogens along with the concentrations of one or more fecal indicators in surface water samples indicate a poor level of microbial quality of surface water, and could represent a significant health risk to users.

Objective

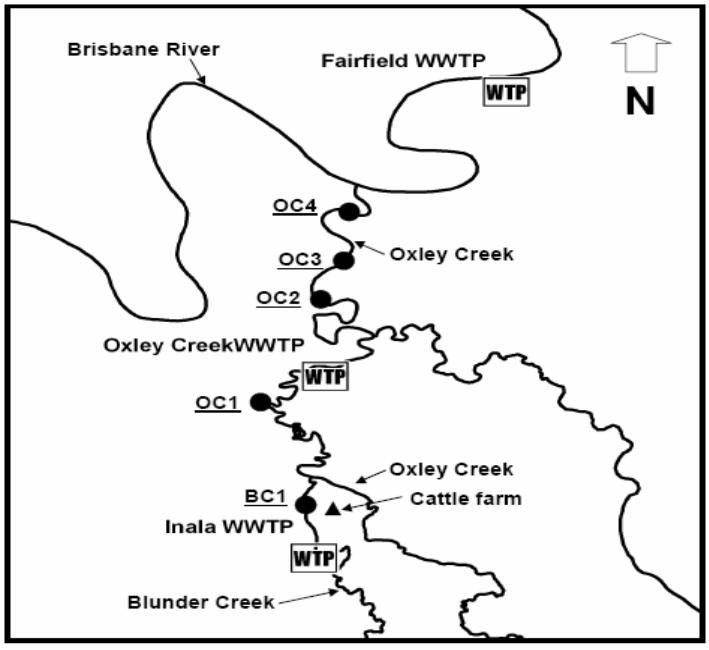
The study investigated the prevalence and concentrations of various zoonotic pathogens in surface waters in Brisbane, Australia using PCR/quantitative PCR (qPCR). Secondly, the correlation between traditional fecal indicator bacteria (i.e., *E. coli* and enterococci) and the selected zoonotic bacterial pathogens that are also commonly found in human sewage were investigated.

Methodology

•Surface water samples were collected from Brisbane City Botanic Gardens (CBG) Pond, and two creeks (i.e., Oxley Creek and Blunder Creek) in Brisbane, Australia. Four sites (OC1-OC4), one site (BC1), and three sites (CBGP1-CBGP3) were selected in Oxley Creek, Blunder Creek and CBG ponds (not shown in the map).

•Membrane filtration method was used for the isolation of *E. coli* and enterococci. For DNA isolation, 500mL aliquots were filtered through membranes and DNA was extracted directly on the membrane using the QIAgen Blood & Tissue kit.

Map: study area and sampling sites



•PCR/qPCR detection of human-specific markers was done using previously published primers and PCR assays

•qPCR standards were prepared from the genomic DNA of control strains. A tenfold dilution was prepared from the genomic DNA, ranging from 10^6 to 10^0 copies/µl of DNA extract, and stored at -20°C until use. For each standard, the concentration was plotted against the cycle number at which the fluorescence signal crossed the threshold value (C*T* value).

•For qPCR, reproducibility, limit of detection, effects of PCR inhibitory substances, recovery efficiency were rigorously evaluated.

•A binary logistic regression analysis was also performed to obtain correlations between the presence/absence of pathogen detection by PCR, and the concentrations of fecal indicators. Logistic regression is the technique most commonly used to model such a binary (i.e., presence/absence) response.

Results

•To detect the presence of inhibitors, surface water samples (n = 3) were spiked with 10^3 gene copies of *S.* Typhimurium DNA containing the *invA* gene. The qPCR *CT* values were compared to those obtained from the same concentrations of DNA that was used to spike 500-ml of distilled water. For the spiked distilled water, the mean *CT* value for *Salmonella invA* gene was 21.6 $\pm$ 0.4. For surface water samples, the mean *CT* values for undiluted DNA, ten-fold, 100-fold and 1000-fold are shown in the Table below. The results indicated that the undiluted DNA extracted from surface water samples contained PCR inhibitory substances. Ten to 100 fold dilution of DNA is required to remove the effects of PCR inhibitory substances from surface water samples.

Samples	Threshold cycle (C_T) value for the qPCR			
	Undiluted DNA	10-fold dilution	100-fold dilution	1000-fold dilution
Surface water 1	37.6 ± 2.6	22.0 ± 0.3	21.7 ± 0.5	21.5 ± 0.1
Surface water 2	34.6 ± 6.1	22.6 ± 1.6	21.6 ± 0.2	21.3 ± 0.5
Surface water 3	31.3 ± 6.5	24.6 ± 3.1	21.6 ± 0.2	21.6 ± 0.1
Mean C_T values	34.5 ± 3.1	23.0 ± 1.3	21.6 ± 0.1	21.4 ± 0.2

•Of the 12 samples tested from the CBG Pond, five (42%) were positive for *C. jejuni mapA* gene. Quantitative PCR detected 3.0 X 10^1 to 7.0 X 10^1 gene copies/100ml of *C. jejuni mapA* gene in these positively identified samples. Of the 12 samples tested from the CBG Pond, only one (8%) was positive for *Salmonella invA* gene, and the concentration was 1.2 X 10^2 gene copies/100ml. However, the *Salmonella invA* could not be detected in any samples from the Oxley Creek or Blunder Creek. Among the 12 samples tested from the CBG Pond, five (42%), three (25%), and five (42%) were positive for EHEC O157 LPS, VT1 and VT2 genes, respectively.

•Overall, of the 32 samples tested, eight (25%), one (3%), nine (28%), 14 (44%) and 15 (47%) were positive for *C. jejuni mapA* gene, *Salmonella invA* gene, *E. coli* O157 LPS, VT1, and VT2 genes, respectively.

•Binary logistic regressions were used to identify whether any correlation existed between the concentrations of fecal indicators and the presence/absence results for potential target pathogens. The presence/absence of the potential pathogens did not correlate with either *E. coli* or enterococci concentrations.

Concentrations of fecal indicators and PCR positive results for zoonotic pathogens

Study area	No. of samples	*E. Coli* (CFU/100 ml)	Enterococci (CFU/100 ml)	*C. Jejuni mapA* gene	*Salmonella invA* gene	O157 LPS	VT1	VT2
CBG Pond	12	370-35,000	800-63000	5/12	1/12	5/32	3/12	5/12
Oxley Creek	16	50-4700	90-1500	2/16	0/16	3/16	9/16	8/16
Blunder Creek	4	<1-110	55-2500	1/4	0/4	1/4	2/4	2/4

Conclusions

•The high prevalence and concentrations of potential zoonotic pathogens along with the concentrations of one or more fecal indicators in surface water samples indicate a poor level of microbial quality of surface water especially after rainfall events, and could represent a significant health risk to users. This underlines the need to undertake appropriate mitigation measures to protect public health risks.

•This study also indicated a poor correlation between fecal indicators and potential zoonotic pathogens tested. Therefore, testing fecal indicators alone may not be adequate to assess the microbiological quality of surface water and consequent health risks. The need to undertake a suite of tests to assess the microbiological quality is recommended.

•The study undertaken was limited in terms of the geographica area. Additionally, the results derived were based on four sampling episodes. It is recommended that more widespread sampling is undertaken to determine the geographical and temporal stability of the methods adopted and to assess the prevalence of the detected pathogens outside the study area within this region.