Quantitative PCR assay of sewage-associated *Bacteroides* markers to assess sewage pollution in an urban lake in Dhaka, Bangladesh

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Abstract: This paper aimed to assess the magnitude of sewage pollution in an urban lake in Dhaka, Bangladesh, by using quantitative PCR of sewage-associated *Bacteroides* HF183 markers. PCR was also used for the quantitative detection of ruminant wastewater-associated CF128 markers along with the enumeration of traditional fecal indicator bacteria, namely enterococci. The number of enterococci in lake water samples ranged from 1.1×10^4 to 1.9×10^5 colony-forming units/100 mL water. From the 20 water samples tested, 14 (70%) and 7 (35%) were PCR positive for HF183 and CF128 markers, respectively. The numbers of HF183 and CF128 markers in lake water samples were 3.9×10^4 to 6.3×10^7 and 9.3×10^3 to 6.3×10^5 genomic units/100 mL water, respectively. The high numbers of enterococci and HF183 markers are indicative of sewage pollution and potential health risks to those who use the lake water for nonpotable purposes such as bathing and washing clothes. This is the first study that investigated the presence of microbial source tracking markers in Dhaka, Bangladesh, where diarrhoeal disease is one of the major causes of childhood mortality. The molecular assay used in this study can provide valuable information on the extent of sewage pollution, thus facilitating the development of robust strategies to minimize potential health risks.

Key words: sewage pollution, fecal indicator bacteria, microbial source tracking, host-specific molecular markers, public health risk.

Résumé : Cet article visait à déterminer l'ampleur de la pollution par les eaux d'égouts d'un lac urbain à Dhaka, au Bengladesh, à l'aide de PCR quantitative (qPCR) des marqueurs HF183 de *Bacteroides* associés aux eaux d'égout. La PCR a aussi été utilisée pour détecter quantitativement la présence des marqueurs CF128 associés aux eaux usées par les ruminants, parallèlement à la numération d'indicateurs fécaux bactériens traditionnels, notamment les entérocoques. Le nombre d'entérocoques des échantillons d'eau du lac s'échelonnait de $1{,}1 \times 10^4$ à $1{,}9 \times 10^5$ UFC/100 mL d'eau. Des 20 échantillons d'eau testés, 14 (70 %) et 7 (35 %) étaient respectivement positifs aux marqueurs HF183 et CF128. Le nombre de marqueurs HF183 et CF128 des échantillons d'eau de lac étaient de $3{,}9 \times 10^4$ à $6{,}3 \times 10^7$ et $9{,}3 \times 10^3$ à $6{,}3 \times 10^5$ unités génomiques/100 mL d'eau, respectivement. Le nombre élevé d'entérocoques et de marqueurs HF183 indique la présence de pollution par les eaux d'égout et un risque potentiel pour la santé de ceux qui utilisent l'eau du lac à des fins autres que la consommation, soit la baignade ou le lavage de vêtements. Il s'agit de la première étude qui s'est penchée sur la présence de marqueurs de suivi des sources microbiennes à Dhaka, au Bengladesh, où la diarrhée est une des causes principales de mortalité infantile. Le dosage moléculaire tel qu'utilisé dans cette étude peut fournir des informations précieuses sur l'ampleur de la pollution par les eaux d'égouts, facilitant ainsi le développement de stratégies robustes afin de minimiser les risques potentiels pour la santé.

Mots-clés : pollution par les eaux d'égout, indicateur fécal bactérien, suivi des sources microbiennes, marqueurs moléculaires spécifiques à l'hôte, risque pour la santé publique.

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Introduction

Fecal pollution is a major concern in relation to water bodies that are used for potable and nonpotable purposes because of possible exposure to a wide array of disease-causing microorganisms (Hörman et al. 2004). It has been estimated that there are approximately 1.5 million deaths per year in developing countries as a result of unsafe drinking water and inadequate sanitation (Prüss-Üstun et al. 2008). A range of point and nonpoint sources have been

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identified in the research literature as potential sources of such pollution (McLellan 2004; Ahmed et al. 2005). Microbiological safety of water is generally assessed by enumerating fecal indicator bacteria such as *Escherichia coli* and enterococci (US Environmental Protection Agency 2000). These indicators are commonly present in the feces of warm-blooded animals, including humans, and therefore do not provide specific information regarding their sources.

The source identification of fecal pollution (i.e., humans vs. animals) in environmental waters is vitally important for implementing appropriate mitigation strategies to minimize public health impacts. Human-sourced fecal pollution poses a great health risk because it results in exposure to a wide array of relevant pathogens and has the lowest threshold of public acceptability. However, the identification and designation of pollution sources in environmental waters is difficult owing to the cosmopolitan nature (i.e., shared by a diversity of warm-blooded animals) of fecal indicator bacteria (Field and Samadpour 2007).

Over the last decade, researchers have developed a range of microbial source tracking tools that can be used to distinguish human-sourced fecal pollution from that of animals (Parveen et al. 1997; Bernhard and Field 2000; Ahmed et al. 2005; Fong et al. 2005; Scott et al. 2005). In recent years, PCR detection of host-specific molecular markers such as anaerobic bacterial gene markers (Bernhard and Field 2000), bacterial toxin gene markers (Khatib et al. 2002), and viral markers (Fong et al. 2005) has gained popularity because of the ability of these markers to identify the sources of fecal pollution in environmental waters. Quantitative PCR (qPCR) methods have also been applied to quantify such markers in environmental waters (Seurinck et al. 2006; Kildare et al. 2007; Reischer et al. 2007). Among these markers, sewage-associated *Bacteroides* are reported to be host specific (i.e., human) and geographically stable. They have been widely used to detect the sources of sewage pollution in developed countries such as the United States (Bernhard et al. 2003), France (Gourmelon et al. 2007), Portugal, United Kingdom, Ireland (Gawler et al. 2007), and Australia (Ahmed et al. 2008). qPCR methods have also been developed to quantify *Bacteroides* markers in environmental waters in Belgium (Seurinck et al. 2006), Hawaii (Betancourt and Fujioka 2006), Japan (Okabe et al. 2007), Austria (Reischer et al. 2007), and the United States (Layton et al. 2006). However, to date none of these microbial source tracking methods has been applied for the identification of fecal pollution sources in developing countries, particularly in Southeast Asian countries such as Bangladesh, where diarrhoeal diseases, namely cholera, due to poor water quality is a serious health concern. The World Health Organization has estimated that in 2002 in Bangladesh, 68 of 1000 deaths were due to diarrhoeal disease, namely cholera, caused by *Vibrio cholerae* (disability-adjusted life year estimates in 2002; World Health Organization 2006). The source of the infection is typically cholera patients, when their untreated fecal materials enter environmental waters or drinking water supplies.

The primary aim of the present study was to assess the magnitude of sewage pollution in an urban lake in Dhaka, Bangladesh, by using qPCR of sewage-associated *Bacteroides* HF183 markers. Water samples were also tested for ruminant wastewater-specific CF128 markers using qPCR along with enumeration of traditional fecal indicator bacteria, namely enterococci, using a culture-based method. The results suggest that the study lake is highly polluted with human-derived sewage and could pose human health risks.

Materials and methods

Host group sampling and DNA extraction

To determine the sensitivity and specificity of HF183 and CF128 markers, 45 fecal samples were collected from 5 host groups, including humans, cattle, dogs, cats, and chickens. Human fecal samples (n = 15) were collected from healthy individuals. Cattle fecal samples (n = 12) were collected from 2 farms located outside the city of Dhaka. Dog (n = 5) and cat (n = 6) fecal samples were collected from different houses in the neighbourhood. Chicken fecal samples (n = 7) were collected from a local poultry market. A fresh fecal sample (approximately 0.2–0.5 g) was collected from each individual animal, transported on ice to the laboratory, and processed within 8 h. DNA was extracted from fresh feces (i.e., 150–200 mg) by using a QIAamp DNA stool kit (Qiagen, Valencia, Calif., USA). Extracted DNA samples were resuspended in 200 μL Buffer AE and stored at –80 °C.

Study lake

The study lake is located between Baridhara Diplomatic Zone and Gulshan residential areas of Dhaka, the capital of Bangladesh (Fig. 1). The approximate coordinates are 23°48′4″N and 90°25′2″E. The lake is approximately 1150 m long and 100 m wide. The local residents use lake water for bathing and washing clothes. The potential sources of pollution include sewage flow via stormwater drains and the discharge of fecal materials from slum-like establishments located along the Gulshan part of the lake. During a sanitary survey, a small number of cattle (n = 2) and wild dogs were observed, which can also be potential sources of fecal pollution. After rainfall (i.e., >100 mm), the lake overflows and submerges surrounding residential areas for a few days because of poor drainage conditions.

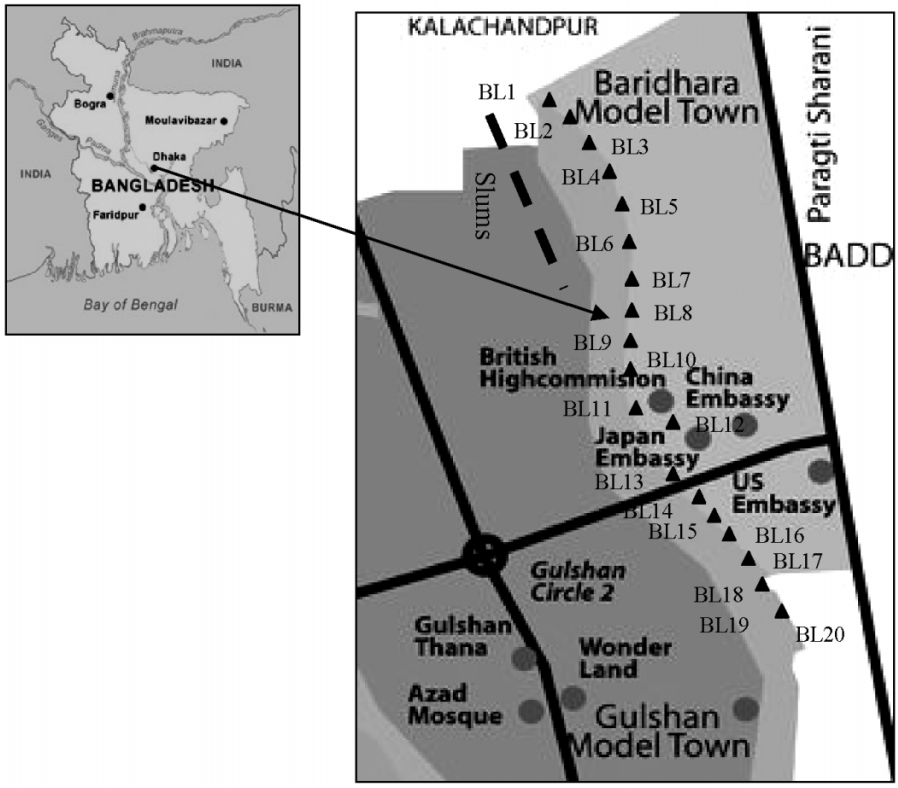
Water sampling

Environmental water samples (n = 20) were collected on 8 August 2009 from 20 locations (sites BL1–BL20) in the lake (Fig. 1). Samples were collected from the bank of the Baridhara Diplomatic Zone because of ease of access. Grab samples were taken from 30 cm below the water surface 2 weeks after a rainfall event >150 mm. The samples were collected in sterile 500 mL plastic containers and immediately transported to the adjacent laboratory at the Independent University of Bangladesh (IUB) for microbiological analysis.

Enumeration of enterococci

The membrane filtration method was used to process the environmental samples for enterococci enumeration. Sample serial dilutions were made, filtered through nitrocellulose membranes (0.45 μm pore size, 47 mm diameter; Advantec, Tokyo, Japan), and placed on membrane-*Enterococcus* indoxyl-D-glucoside (mEI agar) (Difco, Detroit, Mich.,

Fig. 1. A map showing the sampling sites (▲) in Baridhara Lake, Dhaka, Bangladesh.



USA) for the isolation of enterococci. Agar plates were incubated at 41 °C for 48 h. For bacterial enumeration, all samples were tested in triplicate.

DNA extraction

Water samples were processed according to a previously published method (Bernhard and Field 2000). Briefly, 100 mL of water samples were filtered through nitrocellulose membranes (0.45 μm pore size; Advantec), and DNA was extracted by using DNeasy blood and tissue kit (Qiagen). Extracted DNA was resuspended in 200 μL Buffer AE and stored at –80 °C. qPCR analysis was performed at the University of the Sunshine Coast (USC), Sippy Downs, Queensland, Australia. DNA isolated from fecal and water samples was lyophilized prior to transport to USC. At USC, the samples were resuspended in 200 μL Buffer AE (Qiagen) and stored at –20 °C. Prior to analysis, the concentration of DNA was determined using a spectrophotometer.

PCR assays

qPCR was used to quantify HF183 and CF128 markers. HF183 markers were amplified by using a forward primer (5′-ATCATGAGTTCACATGTCCCG-3′) (Bernhard and Field 2000) and a reverse primer (5′-TACCCCGCCTACTATCTAATG-3′) (Seurinck et al. 2006). CF128 markers were amplified using a previously published primer set (forward primer: 5′-CCAACYTTCCCGWTACTC-3′; reverse primer: 5′-CAATCGGAGTTCTTCGTG-3′) (Bernhard and Field 2000). The qPCR mixture contained 12.5 μL of Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, Calif., USA), 300 nmol/L of each primer, and 5 μL of template DNA. The HF183 qPCR consisted of 2 min at 50 °C, 10 min at 95 °C followed by 45 cycles of 30 s at 95 °C, 1 min at 53 °C, and then an extension of 1 min at 60 °C. CF128 qPCR consisted of 2 min at 50 °C, 15 min at 95 °C followed by 40 cycles of 30 s at 94 °C, 30 s at 59 °C, and then an extension of 1 min at 72 °C.

The HF183 and CF128 positive controls were isolated from sewage and cattle wastewater, respectively. The PCR-amplified product was purified using a QIAquick PCR purification kit (Qiagen) and cloned into a pGEM-T Easy Vector system (Promega, Madison, Wis., USA), transferred into *E. coli* JM109-competent cells, and plated on Luria–Bertani agar plates containing ampicillin, isopropyl β-D-1-thiogalactopyranoside (IPTG), and X-gal, as recommended by the manufacturer. Recombinant plasmids with corresponding inserts were purified using a plasmid mini kit (Qiagen). DNA sequencing was carried out at the Australian Genome Research Facility at St. Lucia, Queensland, Australia.

Standards for qPCR of HF183 and CF128 markers were prepared from the plasmid DNA. A 10-fold serial dilution was prepared from the plasmid DNA, ranging from 10^6 to 10^0 copies/μL. The reproducibility of the qPCR assays was assessed by determining intra-assay repeatability and inter-assay variations. The coefficient of variation was calculated using 5 dilutions (10^6–10^2 gene copies) of the HF183 and CF128 plasmid DNA. Each dilution was quantified in triplicate.

For the qPCR standards, the concentration was plotted against the cycle number at which the fluorescence signal increased above the threshold value (C_T value). The amplification efficiency (E) was determined by running the standards and was estimated from the slope of the standard curve by the formula $E = (10^{-1/\text{slope}}) - 1$. A reaction with 100%

efficiency generates a slope of –3.32. To separate the specific product from nonspecific products, including primer dimers, melting curve analysis was performed for each PCR run. In the melting curve analysis, the temperature was increased from 55 to 95 °C at approximately 2 °C/min. Nonspecific products were not observed during the melting curve analysis.

qPCR limit of detection in freshwater

To determine the qPCR limit of detection of HF183 markers, sewage samples were collected from a sewage treatment plant in Brisbane, Australia, and suspended in freshwater collected from the Brisbane River ($n = 3$) at a ratio of 1:1 (62.5 mL water : 62.5 mL fresh sewage). Sample serial dilutions (10^{-1}–10^{-10}) were made, and DNA extraction was performed for each dilution and tested with qPCR. The number of enterococci was enumerated using the membrane filtration method for each dilution, according to the method described above.

Recovery efficiency

The recovery efficiency was determined by spiking autoclaved freshwater samples ($n = 3$) with known numbers of *Bacteroides vulgatus* ATCC 8482. For recovery efficiency, *B. vulgatus* was used because *Bacteroides* species that carry the HF183 and CF128 markers have never been isolated. *Bacteroides vulgatus* was grown overnight according to a previously published method (Seurinck et al. 2006), and cell numbers were determined by using flow cytometry. Tenfold serial dilutions ($2.1 \pm 0.4 \times 10^7$, $2.1 \pm 0.4 \times 10^6$, $2.1 \pm 0.4 \times 10^5$, and $2.1 \pm 0.4 \times 10^4$ cells/100 mL freshwater) of the stationary phase of *B. vulgatus* were made and filtered through the membranes. DNA extraction was performed according to the method described above. For the quantification of *B. vulgatus*, a bacterial qPCR assay was used. The primers and cycling condition of this assay are described elsewhere (Boon et al. 2003). A 10-fold dilution series (ranging from 10^6 to 10^2 copies) of DNA extracted from *B. vulgatus* was used as the qPCR standard for the *B. vulgatus* bacterial assay. The recovery efficiency was calculated using the following equation: Recovery (%) = (No. of cells after filtration / No. of cells before filtration) × 100.

Testing for PCR inhibitors in DNA isolated from environmental waters

An experiment was conducted to determine the potential presence of PCR inhibitory substances in representative DNA samples ($n = 5$) isolated from environmental waters collected from the study lake. DNA samples were serially diluted (i.e., 10-fold and 100-fold), and undiluted, 10-fold, and 100-fold dilutions were spiked with 10^3 gene copies of the ruminant wastewater-associated CF128 *Bacteroides* markers (Bernhard and Field 2000). CF128 markers were chosen, as the prevalence of these markers is assumed to be low in samples collected from the lake compared to prevalence of HF183 markers. Before spiking, all 5 DNA samples were tested to determine the background level of CF128 markers. None of the samples was found to be positive for CF128 markers. The C_T values of the spiked DNA samples were compared to those of the DNA samples from distilled water spiked with 10^3 gene copies of CF128 markers.

DNA sequencing

To verify the identity of the PCR product obtained from lake water samples using HF183 and CF128 *Bacteroides* primers, the PCR-amplified sequences from the HF183 and CF128 primer sets were cloned into the pGEM-T Easy Vector system (Promega). Plasmids were extracted using a QIAprep Spin Miniprep kit (Qiagen). Bidirectional sequences were obtained using T7 and SP6 long sequencing primers targeting sites on either side of the insert. DNA sequencing was carried out and analysed using Jellyfish Software (Field Scientific, Lewisberg, Pa.; http://www.jellyfishsoftware.com).

Statistical analysis

One-way ANOVA was performed to determine the differences between the C_T values obtained for distilled water and those obtained for DNA isolated from surface water samples. Results were considered significant at $P < 0.05$.

Results

Sensitivity and specificity

HF183 markers were detected in 13 of 15 human fecal samples tested and was not detected in 28 of 30 animal fecal samples. However, fecal samples from 1 dog and 1 cat were positive. CF128 markers were detected in 9 of 12 cattle fecal samples and not detected in fecal samples from humans and other animal host groups. The number of HF183 and CF128 markers in positively identified samples is given in Table 1. The overall sensitivity of the HF183 primer in detecting sewage-associated HF183 markers in human fecal samples was 0.87. For CF128 markers, this figure was 0.75. The overall specificity of the HF183 markers to differentiate between sewage and animal feces was 0.93. For CF128, this figure was 1.0.

qPCR standards and reproducibility

Tenfold serial dilutions of HF183 and CF128 plasmid DNA were analysed to determine the reaction efficiencies. The standard curves had a linear range of quantification from 10^6 to 10^1 HF183 and CF128 markers per 1 µL of DNA extract. The amplification efficiencies were between 95% and 99% for both markers. The correlation coefficient (r^2) was >0.97. The amplification of the correct PCR products was verified by analysing the melting curves. The reproducibility of the qPCR assays was determined by assessing the intra-assay and inter-assay coefficient of variation of the standards. These values were less than 2% and 5%, respectively, for both assays, indicating high reproducibility.

Limit of detection and recovery efficiency

Prior to testing environmental samples, the limit of detection of qPCR was evaluated for the HF183 markers. qPCR was able to detect HF183 markers up to a dilution of 10^{-8} in freshwater spiked with sewage. At this dilution, no culturable enterococci were found. The estimated recovery efficiency in freshwater samples ranged from 86% to 50%, with the greatest variability occurring at lower cell counts (Table 2). The mean detection efficiency was 68% ± 10%.

Table 1. PCR results for the number of sewage-associated *Bacteroides* HF183 and ruminant-associated CF128 markers in host groups in Dhaka, Bangladesh.

		HF183			CF128		
Host group	No. of samples	Positive	No. markers/ 100 mg feces	Negative	Positive	No. markers/ 100 mg feces	Negative
Humans	15	13	1.2×10^6–3.9×10^8	2	0	0	15
Cattle	12	0	0	12	9	5.1×10^6–6.3×10^7	3
Dogs	5	1	7.8×10^4	4	0	0	5
Cats	6	1	4.6×10^3	5	0	0	6
Chickens	7	0	0	7	0	0	7

Table 2. Detection efficiency with the qPCR assay for autoclaved freshwater samples with known numbers of *Bacteroides vulgatus* cells.

No. cells/500 mL water	Detection efficiency ± SD (%)
$2.1\pm0.4\times10^7$	80±6
$2.1\pm0.4\times10^6$	73±7
$2.1\pm0.4\times10^5$	66±9
$2.1\pm0.4\times10^4$	46±6

PCR inhibitors

For the spiked distilled water, the mean C_T value for the CF128 marker was 27.3 ± 0.5. For surface water samples, the mean C_T values for undiluted, 10-fold diluted, and 100-fold diluted DNA samples are shown in Table 3. One-way ANOVA was performed to determine the differences between the C_T values obtained for DNA isolated from distilled water and those obtained for surface water samples. However, significant ($P < 0.001$) differences were observed between the C_T values for spiked distilled water DNA and undiluted DNA from lake water samples, indicating that the undiluted DNA extracted from the lake contained PCR inhibitory substances. Significant ($P < 0.001$) differences were also observed between spiked distilled water DNA and 10-fold diluted DNA. However, no significant differences ($P > 0.05$) were observed between the C_T values for spiked distilled water DNA and 100-fold diluted DNA, indicating that a 100-fold dilution of DNA was required to remove the effects of PCR inhibitory substances.

Number of enterococci

The number of enterococci in surface water samples ranged from 1.1×10^4 to 1.9×10^5 colony-forming units (CFU)/100 mL of water. Site BL18 had the lowest number (1.1×10^4 CFU/100 mL), and site BL12 the highest number (1.9×10^5 CFU/100 mL) (Table 4). The number of enterococci was also extremely high at sites BL3 and BL9. These are located near stormwater pipes draining into the lake. All water samples tested exceeded the recommended guideline values for enterococci in fresh and marine waters (US Environmental Protection Agency 1986; World Health Organization 2003).

qPCR results for environmental water samples

Of the 20 samples tested, 14 (70%) were positive for HF183 markers, and the number of markers in these positively identified samples ranged from 3.9×10^4 to 6.3×10^7 genomic units (GU)/100 mL water (Table 4). The highest number (6.3×10^7 GU/100 mL) of markers was found in water samples collected from site BL3 followed by BL6 (6.1×10^7) and BL9 (5.3×10^7). Of the 20 water samples tested, 7 (35%) were PCR positive for the ruminant wastewater-associated markers. The number of CF128 markers in these positively identified samples ranged from 9.3×10^3 to 6.3×10^5 GU/100 mL. In all, 4 (20%) samples were positive for both HF183 and CF128 markers. Up to 3 amplicons (i.e., amplified from environmental DNA) of each marker were sequenced and verified to ensure that they were >97% identical to the published sequence.

Discussion

The high number of HF183 markers in lake water samples indicates the presence of sewage pollution. Stormwater pipes and slum-like establishments in the vicinity may have introduced sewage pollution to the lake. Prior to water sampling, a sanitary survey was undertaken to identify possible sources of fecal pollution other than human sources. A small number of wild dogs and a few cattle were observed near the lake. HF183 markers have been isolated occasionally from the feces of companion animals such as dogs and cats (Ahmed et al. 2008). As such, prior to field application, the specificity of HF183 and CF128 markers was evaluated by testing fecal samples from 5 host groups. It is acknowledged that the number of samples tested in the specificity assay was small. Nonetheless, the HF183 marker displayed high specificity. These markers were only detected in 2 fecal samples from companion animals, and the numbers were low compared to those in human feces (see Table 1). The high specificity of HF183 markers in distinguishing human from animal feces has been reported from many locations around the world (Seurinck et al. 2006; Gourmelon et al. 2007; Ahmed et al. 2008). To rule out the possibility of dog-sourced fecal pollution, dog *Bacteroides* BacCan markers were used to identify dog-sourced fecal pollution in water samples (Kildare et al. 2007). Only 2 samples from sites BL9 and BL10 gave positive signals (data not shown). Therefore, considering the limited number of nonpoint sources of fecal pollution and the high specificity of HF183 markers, the most likely source of the HF183 marker in the lake is considered to be fecal discharges from slum-like establishments and stormwater pipes.

All water samples were screened for HF183 and CF128 markers using qPCR. A significant number of water samples (i.e., 70%) were positive for HF183 markers. However, these markers could not be detected at sites BL17, BL18,

Table 3. Effects of PCR inhibitors on the qPCR detection of spiked ruminant wastewater-associated CF128 *Bacteroides* markers in DNA isolated from surface water samples as opposed to distilled water samples.

	C_T value for qPCR of surface water samples		
Sample	Undiluted DNA	10-fold dilution	100-fold dilution
Distilled water	27.3±0.5	—	—
BL4	35.6±0.8	31.1±0.4	27.3±0.6
BL5	34.9±0.7	30.0±0.6	28.1±0.8
BL6	36.2±0.8	32.1±0.6	27.9±0.6
BL7	35.3±0.6	31.4±0.9	27.6±0.7
BL8	35.1±0.9	31.6±0.6	27.4±0.6

Note: C_T, threshold cycle.

Table 4. Number of enterococci and sewage-associated *Bacteroides* HF183 and ruminant wastewater-associated *Bacteroides* CF128 markers in surface water samples collected from Baridhara Lake, Dhaka, Bangladesh.

Sampling site	No. of enterococci (CFU/100 mL)	No. of sewage-associated *Bacteroides* HF183 markers/100 mL	No. of ruminant wastewater-associated *Bacteroides* CF128 markers/100 mL
BL1	9.0×10^4	3.3×10^6	1.2×10^4
BL2	8.0×10^4	2.1×10^5	nd
BL3	1.8×10^5	6.3×10^7	3.1×10^4
BL4	5.2×10^4	2.1×10^7	nd
BL5	4.0×10^4	8.9×10^6	nd
BL6	5.5×10^4	6.1×10^7	nd
BL7	6.0×10^4	2.1×10^6	nd
BL8	3.5×10^4	4.8×10^5	nd
BL9	1.3×10^5	5.3×10^7	5.1×10^4
BL10	3.2×10^4	9.1×10^5	nd
BL11	2.2×10^4	nd	nd
BL12	1.9×10^5	5.1×10^7	6.3×10^5
BL13	2.2×10^4	1.1×10^5	nd
BL14	4.7×10^4	3.9×10^4	nd
BL15	1.9×10^4	nd	nd
BL16	5.6×10^4	1.0×10^5	nd
BL17	2.6×10^4	nd	3.7×10^4
BL18	1.1×10^4	nd	9.3×10^3
BL19	2.0×10^4	nd	nd
BL20	1.4×10^4	nd	7.3×10^4

Note: nd, not detected. CFU, colony-forming units.

BL19, and BL20, possibly because these sites were located approximately 1 km from the suspected sources of sewage pollution. CF128 markers were detected in 7 samples; however, the numbers were much lower than those found in cattle feces.

The number of HF183 markers in human feces and sewage (collected from influent) ranged from $8.4 \pm 0.1 \times 10^5$ to $7.2 \pm 1.1 \times 10^9$/g wet feces and $5.9 \pm 0.7 \times 10^9$ to $3.1 \pm 0.3 \times 10^{10}$ GU/L, respectively (Seurinck et al. 2006). Similar numbers of HF183 have been detected in feces collected from humans in the present study (see Table 1). In this study, the number of HF183 markers in lake water samples ranged from 3.9×10^4 to 6.3×10^7 GU/100 mL water, which is 1–2 orders of magnitude lower than that found in human feces or sewage. Such data are cause for significant concern and indicate the presence of sewage pollution. The number of enterococci was also high, indicating a high level of fecal pollution. The number of enterococci was higher near the stormwater pipes, thus confirming that these pipes are contributing to the fecal load in the lake. It has to be noted that the samples were collected after a rainfall event, when the fecal indicator numbers are expected to be high in environmental waters. Nonetheless, the high levels of fecal indicator bacteria and HF183 *Bacteroides* markers indicate sewage pollution and health risks to those who use the lake water for nonpotable uses such as bathing and washing clothes. The overflow from the lake after heavy rain could also pose serious health risks to the residents surrounding the lake because of the possibility of cross-contamination with the reticulated supply water. The significant increase in diarrhoeal cases after flood or storm events are common in Bangladesh (Hashizume et al. 2008).

The qPCR assays as employed in this study could provide a scientifically reliable approach to source tracking and for

assessing the magnitude of fecal pollution within a short time frame. The qPCR assays also generate valuable data that could be used to develop strategies to minimize fecal pollution or, at the minimum, to educate local residents regarding the health risks associated with exposure to such pollution. Local laboratories could employ such methods for rapid assessment of sewage pollution in environmental waters. In the near future we intend to evaluate the specificity of HF183 markers thoroughly by testing more animal species as well as testing more water samples from the study area at different time periods.

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