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Monitoring of Waterborne Pathogens in Surface Waters in Amsterdam, The Netherlands, and the Potential Health Risk Associated with Exposure to *Cryptosporidium* and *Giardia* in These Waters[▽]

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The water in the canals and some recreational lakes in Amsterdam is microbiologically contaminated through the discharge of raw sewage from houseboats, sewage effluent, and dog and bird feces. Exposure to these waters may have negative health effects. During two successive 1-year study periods, the water quality in two canals (2003 to 2004) and five recreational lakes (2004 to 2005) in Amsterdam was tested with regard to the presence of fecal indicators and waterborne pathogens. According to Bathing Water Directive 2006/7/EC, based on *Escherichia coli* and intestinal enterococcus counts, water quality in the canals was poor but was classified as excellent in the recreational lakes. *Campylobacter*, *Salmonella*, *Cryptosporidium*, and *Giardia* were detected in the canals, as was rotavirus, norovirus, and enterovirus RNA. Low numbers of *Cryptosporidium* oocysts and *Giardia* cysts were detected in the recreational lakes, despite compliance with European bathing water legislation. The estimated risk of infection with *Cryptosporidium* and *Giardia* per exposure event ranged from 0.0002 to 0.007% and 0.04 to 0.2%, respectively, for occupational divers professionally exposed to canal water. The estimated risk of infection at exposure to incidental peak concentrations of *Cryptosporidium* and *Giardia* may be up to 0.01% and 1%, respectively, for people who accidentally swallow larger volumes of the canal water than the divers. Low levels of viable waterborne pathogens, such as *Cryptosporidium* and *Giardia*, pose a possible health risk from occupational, accidental, and recreational exposure to surface waters in Amsterdam.

Exposure to microbiologically contaminated surface water may have adverse health effects and may result in gastroenteritis (GE); fever; skin, ear, and eye complaints; or more severe illnesses, such as hepatitis and meningitis (61). Protozoan parasites have frequently been the cause of water-associated outbreaks in Europe (33). The surveillance system in the United States has detected numerous outbreaks of disease associated with recreational waters over the years in which pathogens such as *Cryptosporidium*, *Giardia*, and norovirus were regularly identified as the etiological agent (20). Illness as a result of infections due to recreational water contact is difficult to detect and to attribute to water exposure (61). In The Netherlands, records of health complaints possibly related to recreational water demonstrate that each year over 50% of the authorities responsible for recreational water quality are aware of water-related health complaints, the majority comprising GE and skin conditions (48). Microorganisms in surface waters may originate from several sources. In The Netherlands, it has been demonstrated that pathogenic microorganisms may enter surface waters through discharges of raw and treated sewage and manure runoff from agricultural land (55, 37).

In Amsterdam, particularly during summer, people jump, fall, or get pushed into the canals and thus are exposed to the canal water. Other people, like professional divers, are exposed to canal water when engaged in their profession. The Amsterdam canals are not official European bathing sites, and therefore, water quality is not routinely monitored. However, Amsterdam Municipal Health Services is aware of contamination of the water in Amsterdam canals by sewage discharge from houseboats in the canals that are not connected to the sewer system, effluents from sewage treatment plants in the vicinity of Amsterdam transported into the canals by the river Amstel, runoff of dirt from the streets (including dog feces), and direct fecal droppings of birds. Several recreational lakes within the city boundaries of Amsterdam are official bathing sites at which water quality is tested as required by the European Bathing Water Directive (1, 12). Bathing water profiles (12) have indicated that water quality in five of these lakes is affected by the discharge of raw sewage from boats and houseboats, sewage effluent, dog feces on the beaches, and direct fecal droppings from birds.

The presence of waterborne pathogens in bird feces has frequently been reported. In Europe, *Campylobacter jejuni* has been detected in gull (*Larus* spp.) feces in Northern Ireland (41) and Sweden (15, 60). Birds may also contribute to the parasite load of recreational waters. *Cryptosporidium* and *Giardia* have been detected in goose feces in the United States (26) and in gull feces in Scotland (52) and the Czech Republic (44). *Giardia* cysts have been found in the feces of wild ducks

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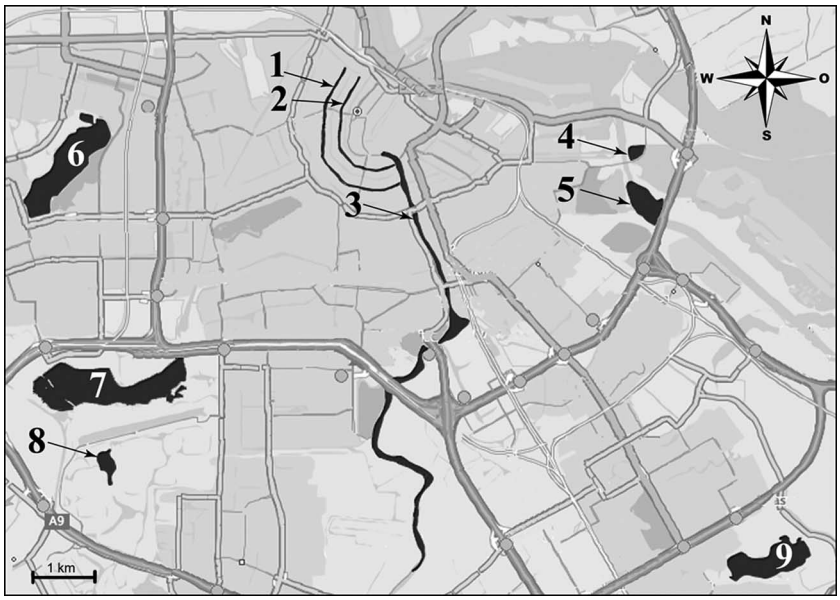

FIG. 1. Nonrecreational sampling sites (Prinsengracht [1], Herengracht [2], Amstel [3], and IJmeer [4]) and recreational sampling sites (Nieuwe Diep [5], Sloterplas [6], Nieuwe Meer [7], Amsterdamse Bos [8], and Gaasperplas [9]) in Amsterdam.

(*Anas* spp.) in New Mexico (34). In Finland, *Cryptosporidium* oocysts and *Giardia* cysts were detected in dog feces (46), whereas a Canadian study showed the presence of *Giardia* cysts and *Cryptosporidium* antibodies in fecal samples from dogs (51).

Previous studies have demonstrated an increase in the number of fecal indicators in surface waters following heavy rainfall events, due to sewage overflow and surface runoff (24, 45). These data suggest that heavy rainfall events may contribute to surface water contamination in Amsterdam and may give rise to increased concentrations of pathogens in the water.

Despite the awareness of sources possibly contributing to surface water contamination in Amsterdam, no data were available on the water quality in the Amsterdam canals and the occurrence of pathogenic organisms in both the canals and recreational lakes. Therefore, this study aimed at testing surface water in Amsterdam intended for recreational and nonrecreational purposes for the presence of a range of waterborne pathogens and compliance with the standards for microbiological quality as required by the 1976 European Bathing Water Directive (1) as well as the revised directive which came into force in 2006 (12). Obtained data were used to provide a rough estimate of the risk of infection with *Cryptosporidium* and *Giardia* from occupational and accidental exposure to canal water and the recreational use of lakes in Amsterdam.

MATERIALS AND METHODS

Sampling sites. Water from a canal with houseboats (Prinsengracht), a canal without houseboats (Herengracht), and the River Amstel and the IJmeer (Fig. 1) was sampled eight times from June 2003 to June 2004. Water from the recreational lakes Sloterplas, Amsterdamse Bos, Gaasperplas, Nieuwe Meer, and Nieuwe Diep (Fig. 1) was sampled 11 times from September 2004 to September 2005. Samples were taken and handled according to ISO 5667-2 (3); sample volumes of approximately 40 liters were collected in polypropylene vessels. All samples were tested for the presence of fecal coliforms, *Escherichia coli*, fecal streptococci, and intestinal enterococci. Samples from the canals and the Amstel and the IJmeer were also examined for F-specific bacteriophages, somatic coliphages, *Campylobacter*, *Salmonella*, *Escherichia coli* O157, *Cryptosporidium*, *Giardia*, rotavirus, enterovirus, reovirus, norovirus, astrovirus, and hepatitis A and E viruses. Samples from the lakes were additionally examined for the presence of *Cryptosporidium* and *Giardia*.

Analytical procedures. (i) Fecal indicator bacteria. The fecal indicator parameters included in European Bathing Water Directive 76/160/EEG, fecal coliforms and fecal streptococci, were enumerated according to Dutch standards (2, 4), using lauryl sulfate agar (M500.02; Tritium Microbiology, Veldhoven, The Netherlands) and KF streptococcus agar (76.4 g KF streptoccus agar [BD Difco 249610; BD Benelux, Breda, The Netherlands], 2 ml 5% 2,3,5-triphenyltetrazolium chloride solution [SR0211; Oxoid Ltd., Basingstoke, United Kingdom], and 1 liter distilled water, prepared according to the manufacturer's instructions) as isolation media, respectively. Fecal indicator parameters included in European Bathing Water Directive 2006/7/EC, *E. coli* and intestinal enterococci, were enumerated by using the rapid test on tryptone soy agar (P05012A; Oxoid, Wesel, Germany) and tryptone bile agar (P05017; Oxoid) described in ISO 9308-1 (6) and according to ISO 7899-2 (7) on Slanetz and Bartley agar (P05018A; Oxoid), respectively.

(ii) Bacteriophages. F-specific bacteriophages were enumerated according to ISO 10705-1 (5) and grown on tryptone yeast extract glucose agar (20 g Bacto Agar [BD 214010; BD Benelux], 8 g sodium chloride [1.06404; Merck KGaA, Darmstadt, Germany], 10 g trypticase peptone [BD 211921; BD Benelux], 1 g yeast extract [Lp0021; Oxoid Ltd.], and 1 liter distilled water, prepared according to ISO 10705-1). Somatic coliphages were enumerated according to ISO 10705-2 (8) and grown on modified Scholtens agar with nalidixic acid (20 g Bacto Agar [BD 214010; BD Benelux], 10 g peptone [Lp0034; Oxoid Ltd.], 12 g Lab Lemco powder [Lp0029; Oxoid Ltd.], 3 g sodium chloride [1.06404; Merck KGaA], 5 ml Na_2CO_3 solution at 150 g/liter [1.00392; Merck KGaA], 0.3 ml $MgCl_2 \cdot 6H_2O$ solution at 2 g/ml [1.05833; Merck KGaA], 250 mg nalidixic acid [190246; ICN Biomedicals Inc., Costa Mesa], and 1 liter distilled water, prepared according to ISO 10705-2).

(iii) *Cryptosporidium* and *Giardia*. For enumeration of *Cryptosporidium* and *Giardia*, water samples (approximately 20 liters) were concentrated by using Envirochek HV filtration capsules (Pall Gelman Laboratory, Ann Arbor, MI) as described in ISO 15553 (11). Concentrated samples were purified by immunomagnetic separation using the Dynal GC-Combo system (Dynal Biotech ASA, Oslo, Norway) according to the manufacturer's instructions. Slides for microscopy were stained with 50 μl *Cryptosporidium* and *Giardia* staining reagent without Evans blue (Cellabs Diagnostics, Brookvale, Australia) at 37°C for 45 min in the dark; subsequently, 5 μl of a propidium iodide solution (PI) (1 mg/ml in phosphate-buffered saline [0.01 M, pH 7.2] was added and incubated for 2 min at room temperature. Slides were subsequently washed with phosphate-buffered

TABLE 1. Standards for fecal indicators according to European Bathing Water Directive 76/160/EEC and revised European Bathing Water Directive 2006/7/EC

Directive	Water type	Parameter	Excellent quality (CFU/100 ml)	Good quality (CFU/100 ml)	Sufficient quality (CFU/100 ml)
76/160/EEC	All	Total coliforms	500	10,000	
		Fecal coliforms	100	2,000	
		Fecal streptococci	100		
2006/7/EC	Inland waters	*Escherichia coli*	500[a]	1,000[a]	900[b]
		Intestinal enterococci	200[a]	400[a]	330[b]
	Coastal waters and transitional waters	*Escherichia coli*	250[a]	500[a]	500[b]
		Intestinal enterococci	100[a]	200[a]	185[b]

[a] Based upon a 95th-percentile evaluation.
[b] Based upon a 90th-percentile evaluation.

saline, dried with a medium-warm hairdryer, mounted with DABCO-glycerol mounting medium, sealed with colorless nail polish, and examined at ×250 magnification using epifluorescence microscopy (Zeiss Axioskop; Carl Zeiss, Jena, Germany). (Oo)cysts were examined in detail by using Nomarski differential interference contrast at ×1,000 magnification to verify the presence of internal structures. (Oo)cysts taking up PI and staining red were considered dead, whereas *Cryptosporidium* oocysts that excluded PI and contained sporozoites (18) and *Giardia* cysts that excluded PI and had nongranular cytoplasm were considered viable (54).

(iv) Viruses. For virus detection, water samples (approximately 20 liters) were concentrated by a conventional filter adsorption-elution procedure (57) and then separated by a modified two-phase method (36). Samples were further concentrated and purified by spin column gel chromatography and ultrafiltration in a Centricon microconcentrator (36). RNA extraction was performed according to Boom et al. (13) with slight modifications (36). Semiquantitative reverse transcription-PCR assays were used to detect the presence of enterovirus, rotavirus, norovirus, astrovirus, and hepatitis A and E virus RNA. Enterovirus detection was performed according to Schwab et al. (50) using primer pair Entero1 and Entero2. Rotaviruses were detected by using primer pair VP6-3 and VP6-4 (59). Norovirus detection was done according to Vennema et al. (58) using the modified primer pair JV12Y and JV13i. For detection of astrovirus, the method described by Guix et al. (27), applying primers A2 and A1, was used. Primer pairs HAV240 and HAV68 were used for hepatitis A detection (14). Detection of hepatitis E virus was performed according to van der Poel et al. (56) and used primer pair Orf2-S1 and Orf2-A1.

A fraction of the concentrated water samples, obtained as described above, was used to inoculate monolayers of buffalo green monkey cells. The assay was performed as described by Lodder and de Roda Husman (37).

(v) *Campylobacter*. The presence or absence of *Campylobacter* in 1-liter volumes was determined by using the method described in ISO 17995 (10). The primary selective enrichment medium was Preston broth (12.5 g nutrient broth no. 2 [CM0067; Oxoid Ltd.], 475 ml distilled water, 25 ml lysed horse blood [SR0048; Oxoid Ltd.], 1 vial *Campylobacter* growth supplement [SR0232; Oxoid Ltd.], and 1 vial Preston selective supplement [SR0117; Oxoid Ltd.], prepared according to ISO 17995), and Karmali agar (P05041A; Oxoid) was used as the secondary growth medium. Typical colonies were examined for the characteristic spiral shape and corkscrew-like motility of *Campylobacter* by microscopy.

(vi) *Salmonella*. The presence or absence of *Salmonella* in 1-liter volumes was determined according to ISO 6579 (9). Buffered peptone water (K168; bioTrading Benelux BV, Mijdrecht, The Netherlands) was used as primary enrichment broth, and Rappaport Vassiliadis soya peptone broth (26.75 g [CM0866; Oxoid Ltd.] and 1 liter distilled water, prepared according to the manufacturer's instructions) as secondary selective enrichment broth. Brilliant green agar (P05033A; Oxoid) was used as selective solid culture medium. Typical colonies were confirmed on urea agar (U010.86.0008; Tritium Microbiology) and triple sugar iron agar (T352.26.0008; Tritium Microbiology) and in lysine decarboxylation medium (L401.25.0005; Tritium Microbiology). Colonies displaying results characteristic for *Salmonella* were typed by the National Reference Laboratory for *Salmonella* at the Laboratory for Infectious Diseases and Perinatal Screening of the RIVM.

(vii) *Escherichia coli* O157. For molecular detection of *E. coli* O157, approximately 500-ml volumes were filtered through 0.4-μm-pore-size polycarbonate membrane filters (Isopore; Millipore, Billerica, MA). DNA was extracted by using a DNeasy tissue kit (Qiagen Benelux BV, Venlo, The Netherlands) according to the manufacturer's instructions. Real-time PCR assays using a LightCycler real-time PCR device were performed to detect the *rfbE* gene present in *E. coli* O157 as described previously (47).

(viii) European Bathing Water Directive. Compliance with the 1976 European Bathing Water Directive requires that 80% of the samples taken during a bathing season (n = 11 or 12) meet the quality standards for the fecal indicators as outlined in Table 1. Moreover, *Salmonella* and enteroviruses must be absent in 1 liter and 10 liters, respectively. The revised 2006 directive distinguishes separate standards for coastal and fresh waters. At least four observations obtained during the current bathing season, supplemented with observations from previous bathing seasons (total n = 16), should be tested for compliance with the standards for fecal indicators (Table 1).

(ix) Precipitation. Rainfall and rainfall intensity data on sampling days and the 3 days preceding sampling were obtained from the Royal Netherlands Meteorological Institute (www.knmi.nl). For all microbiological parameters at all sampling sites, the correlation coefficient between the observed numbers and the amount of rainfall and rainfall intensity was calculated by using the CORREL function in Excel (Microsoft, version 2003).

(x) Risk assessment. For both professional and accidental contact with canal water and recreational use of the studied lakes in Amsterdam, the risk of infection with *Cryptosporidium* and *Giardia* per exposure event was estimated (30). The range of estimated ingested volumes per contact event used in the calculations was based on the results obtained in a survey of diving behavior and water ingestion among occupational and sport divers (49) and the outcome of a study on water ingestion by swimmers in an indoor swimming pool (23). The risk of infection was estimated by using the exponential dose-response model (28) for which $P_{inf} = 1 - e^{-r\mu}$, where P_{inf} is the probability of infection, and the dose $\mu = CV$ (where C is the measured concentration of viable [oo]cysts in the water samples [n/liter] and V is individual consumption of water [liters]. Dose-response parameter values ($r_{Cryptosporidium} = 0.0040$ and $r_{Giardia} = 0.0199$) were used (53). Calculations were done using Mathematica (Wolfram Inc., version 5.1.0).

RESULTS

Fecal indicators and bacteriophages. *E. coli*, fecal coliforms, intestinal enterococci, and fecal streptococci were detected in the majority of the samples from all sites. Concentrations varied throughout the sampling years and per sampling site but were generally lower in the recreational lakes than in the canals and the Amstel and the IJmeer; arithmetic means, medians, and concentration ranges are displayed in Table 2. Somatic coliphages were detected in all samples from the canals, the Amstel, and the IJmeer, except one from the last site (Table 2). Concentrations varied throughout the year and were highest in the Amstel and lowest in the IJmeer. F-specific phages occurred at much lower concentrations (Table 2).

In the canals and the Amstel, the fecal indicator parameters, except *E. coli*, and both somatic and F-specific phages displayed peak concentrations on 15 December 2003, which coincided with heavy rainfall events on this sampling day and 3

TABLE 2. Fecal indicator bacteria and bacteriophages in surface waters in Amsterdam[a]

Site[c]	Quantity	FC (no./100 ml)	FS (no./100 ml)	EC (no./100 ml)	IE (no./100 ml)	FP[b] (no./ml)	SC[b] (no./ml)
Nonrecreational sites							
IJmeer	Mean	575	112	1,928	127	0.06	2.2
	Median	400	50	200	100	0	1.8
	Range	100–1,900	0–400	8–10,000	0–425	0–0.3	0–5.4
Amstel	Mean	1,550	300	1,404	140	1.4	21
	Median	1,350	100	450	100	0.4	18
	Range	400–4,200	0–1,500	28–6,300	35–350	0.1–5.3	2.3–54
Herengracht	Mean	1,838	225	1,552	158	2.2	16
	Median	550	100	338	155	0.1	6.3
	Range	100–9,000	0–1,200	40–8,100	0–350	0–0.94	0.7–54
Prinsengracht	Mean	1,462	212	1,354	148	2.6	13
	Median	450	100	475	160	0.2	5.6
	Range	300–6,000	0–700	80–7,000	90–200	0–14	0.9–45
Recreational sites							
Amsterdamse Bos	Mean	35	71	130	33	ND	ND
	Median	18	33	50	2		
	Range	0–160	2–400	0–800	0–200		
Gaasperplas	Mean	12	8	41	5	ND	ND
	Median	16	10	24	0		
	Range	0–28	0–16	0–110	0–30		
Nieuwe Diep	Mean	33	75	74	72	ND	ND
	Median	14	38	80	6		
	Range	0–130	0–400	0–140	0–600		
Sloterplas	Mean	45	105	122	42	ND	ND
	Median	20	48	100	10		
	Range	0–230	8–500	0–400	0–190		
Nieuwe Meer	Mean	8	27	34	3	ND	ND
	Median	6	18	20	0		
	Range	0–30	0–90	0–160	0–14		

[a] FC, fecal coliforms; FS, fecal streptococci; EC, *E. coli*; IE, intestinal enterococci; FP, F-specific bacteriophages; SC, somatic coliphages.
[b] ND, not done.
[c] Nonrecreational sites were sampled eight times from June 2003 to June 2004; recreational sites were sampled 11 times from September 2004 to September 2005.

days before sampling (25.5 mm in 4 days; average intensity, 1.42 mm/h). *E. coli* concentrations in the canals, the Amstel, and the IJmeer peaked on 27 October 2003; the total amount of rainfall on this day and the preceding 3 days was 10.7 mm; the average intensity was 1.35 mm/h. Correlations between fecal indicator and bacteriophage concentrations and rainfall intensity were average to strong, with correlation coefficients from 0.5 to 0.9.

Fecal indicator concentrations in the recreational lakes varied throughout the sampling year and showed a clear peak at all sites on 7 July 2004. The total amount of rainfall on this day and the preceding 3 days was 57.3 mm; the average rainfall intensity was 7.0 mm/h. Correlations between fecal indicator concentrations and rainfall amount and intensity varied strongly (−0.3 to 0.9), and there was no common pattern for all recreational sites.

Compliance with European bathing water legislation. The water quality at none of the studied nonrecreational sites in Amsterdam complied with the standards for excellent water quality in Bathing Water Directive 76/160/EEC. Water quality in the IJmeer and the Prinsengracht canal was, however, good, but standards for good quality were not met in the Herengracht canal due to high numbers of fecal indicators and the presence of culturable enteroviruses and *Salmonella* and in the Amstel due to the presence of *Salmonella*. The water quality at these sites did not meet the standards for acceptable bathing water quality as required by revised European Bathing Water Directive 2006/7/EC and was therefore classified as "poor." It should, however, be noted that compliance with European bathing water standards was assessed by using data collected throughout a year and not by using data obtained during the bathing season only. Also, fewer observations ($n = 8$) than required by the directives were obtained and used for compliance testing.

The water quality in the Amsterdam recreational lakes complied with the standards for excellent water quality, according to both Bathing Water Directive 76/160/EEC and 2006/7/EC. The required number of samples ($n = 11$) was used to test for compliance with Directive 76/160/EEC; however, this is fewer observations than required for compliance testing with Directive 2006/7/EC.

Pathogenic viruses and bacteria in canals, the Amstel, and the IJmeer. Astroviruses and hepatitis A and E viruses were not found in any of the samples, whereas rotavirus, norovirus, and enterovirus RNA was detected in several samples from all sites (Table 3). Culturable enteroviruses were found in one sample from the canal Herengracht at a concentration of 3.2 PFU per liter. Samples taken from the Amstel and the Herengracht and Prinsengracht canals on 15 December 2003 and 9 February 2004 contained culturable reoviruses. Concentrations were 25 to 37 PFU/liter in the Amstel, 36 to 42 PFU/liter in the Herengracht canal, and 18 to 19 PFU/liter in the Prinsengracht canal. The sample taken from the Herengracht canal on 7 June

TABLE 3. Enteric viruses in surface water at four nonrecreational sites in Amsterdam from June 2003 to June 2004

Site	No. of positive samples/total no. of samples				
	Norovirus RNA	Rotavirus RNA	Enterovirus RNA	Culturable enterovirus	Culturable reovirus
IJmeer	1/8	3/8	4/8	0/8	0/8
Amstel	4/8	7/8	7/8	0/8	2/8
Herengracht	2/8	7/8	3/8	1/8	3/8
Prinsengracht	2/8	5/8	2/8	0/8	2/8

2004 contained 3.3 PFU/liter culturable reovirus. No culturable viruses were detected in water from the IJmeer.

Salmonella enterica serovar Newport and *Salmonella enterica* serovar Virchow were isolated from the Amstel on 15 December 2003 and 9 February 2004, respectively. *Salmonella* serovar Typhimurium phagetype 690 was isolated from the Herengracht canal on 9 February 2004. *Salmonella* was not detected in samples from the IJmeer or the Prinsengracht canal.

Campylobacter was found in three of seven samples from the IJmeer and the Amstel and in six of seven samples from the Herengracht and Prinsengracht canals. *E. coli* O157 was not detected in any of the samples.

Protozoan parasites. *Cryptosporidium* was found at all nonrecreational sites, but the detection frequencies and concentrations varied (Table 4). Detection frequency was lowest in the IJmeer and highest in the Prinsengracht canal. Concentrations in positive samples were generally low, ranging from one to seven viable oocysts per 10 liters, with one outlier of 29 oocysts/10 liters (Table 4). *Giardia* cysts were detected in all of these samples except one from the IJmeer. The number of viable *Giardia* cysts in positive samples was higher than the number of viable *Cryptosporidium* oocysts and ranged from 1 to 157 cysts per 10 liters (Table 4). For all sites except the Prin-

TABLE 4. Protozoan parasites in surface waters in Amsterdam

Site[a]	Type of quantity	*Cryptosporidium*			*Giardia*		
		No. of positive samples/total samples	Total count (no. of oocysts/ 10 liters)	Viable count (no. of oocysts/ 10 liters)	No. of positive samples/total samples	Total count (no. of cysts/ 10 liters)	Viable count (no. of cysts/ 10 liters)
Nonrecreational sites							
IJmeer		2/8			7/8		
	Mean		0.4	0.2		35	30
	Median		0	0		34	16
	Range		0–2	0–1		0–105	0–84
Amstel		5/8			8/8		
	Mean		2.4	1.2		68	53
	Median		1	0		60	44
	Range		0–9	0–5		8–157	7.2–157
Herengracht		5/8			8/8		
	Mean		2.2	1		45	37
	Median		1	0		27	48
	Range		0–10	0–7		1–118	0–94
Prinsengracht		6/8			8/8		
	Mean		5.7	4.4		70	55
	Median		2.6	0.5		69	55
	Range		0–29	0–29		2–167	2–134
Recreational sites							
Amsterdamse Bos		4/11			0/11		
	Mean		0.5	0		0	0
	Median		0	0		0	0
	Range		0–2	0		0	0
Gaasperplas		2/11			3/11		
	Mean		0.6	0.6		0.4	0
	Median		0	0		0	0
	Range		0–4	0–4		0–2	0
Nieuwe Diep		1/11			6/11		
	Mean		0.1	0		2.5	1.2
	Median		0	0		1	0
	Range		0–1	0		0–11	0–5
Sloterplas		5/11			6/11		
	Mean		1.4	0.3		1.1	0.3
	Median		0	0		1	0
	Range		0–12	0–1		0–4	0–4
Nieuwe Meer		2/11			5/11		
	Mean		0.3	0.2		2.2	1.2
	Median		0	0		0	0
	Range		0–2	0–1		0–8	0–8

[a] Nonrecreational sites were sampled eight times from June 2003 to June 2004; recreational sites were sampled 11 times from September 2004 to September 2005.

TABLE 5. Risk of infection with *Cryptosporidium* or *Giardia* at exposure to recreational and nonrecreational water in Amsterdam, assuming ingestion of different volumes of water and taking into account the mean and maximum detected parasite concentrations

Site	Pathogen	Infection risk (%) at ingested vol (ml) of[d]:					
		5.7[a]		16[b]		37[c]	
		Mean	Max	Mean	Max	Mean	Max
Nonrecreational sites							
IJmeer	*Cryptosporidium*	0.00006	0.0002	0.0002	0.0006	0.0004	0.002
Amstel		0.0003	0.001	0.0008	0.003	0.002	0.007
Herengracht		0.0002	0.002	0.0006	0.004	0.002	0.01
Prinsengracht		0.001	0.007	0.003	0.02	0.006	0.04
IJmeer	*Giardia*	0.03	0.09	0.09	0.3	0.2	0.6
Amstel		0.06	0.2	0.2	0.5	0.4	1.2
Herengracht		0.04	0.1	0.1	0.3	0.3	0.7
Prinsengracht		0.06	0.2	0.2	0.4	0.4	1.0
Recreational sites							
Amsterdamse Bos	*Cryptosporidium*	0	0	0	0	0	0
Gaasperplas		0.0002	0.0009	0.0004	0.003	0.0009	0.006
Nieuwe Diep		0	0	0	0	0	0
Sloterplas		0.00008	0.0002	0.0002	0.0006	0.0005	0.002
Nieuwe Meer		0.00004	0.0002	0.0001	0.0006	0.0003	0.002
Amsterdamse Bos	*Giardia*	0	0	0	0	0	0
Gaasperplas		0.0001	0.001	0.0003	0.003	0.0007	0.007
Nieuwe Diep		0.001	0.007	0.004	0.02	0.009	0.04
Sloterplas		0.0004	0.001	0.001	0.003	0.003	0.007
Nieuwe Meer		0.001	0.009	0.004	0.03	0.009	0.06

[a] Average volume ingested by occupational divers in fresh water (49).
[b] Average volume ingested by adult swimmers in swimming pools (23).
[c] Average volume ingested by nonadult swimmers in swimming pools (23).
[d] Max, maximum.

sengracht canal, there was a moderate-to-high correlation between the number of (oo)cysts and the amount of rainfall (correlation coefficient 0.5 to 0.9); the correlation with rainfall intensity was moderate (correlation coefficient 0.4 to 0.7). *Cryptosporidium* oocysts were found in all studied recreational lakes in Amsterdam but with a low frequency (Table 4); in positive samples, numbers ranged from one to four viable oocysts per 10 liters. *Giardia* cysts were detected in all lakes except the Amsterdamse Bos. The detection frequency was also low, and in positive samples, numbers ranged from one to eight viable cysts per 10 liters (Table 4).

Risk assessment. Estimated average volumes of water ingested by occupational divers in fresh water (5.7 ml) (49) and by adult (16 ml) and nonadult (37 ml) swimmers in a swimming pool (23) were used to calculate the risk of infection with *Cryptosporidium* and *Giardia* for an exposed individual.

The infection risk per exposure event at ingestion of 5.7 to 37 ml ranges from 0.00006% to 0.006% for average detected *Cryptosporidium* concentrations at the four studied nonrecreational sites in Amsterdam (Table 5). For *Giardia*, the infection risk ranges from 0.03% to 0.4% (Table 5). For all sites and ingested volumes of 5.7 to 37 ml, maximum *Cryptosporidium* concentrations result in an infection risk of 0.0002 to 0.04%, whereas maximum *Giardia* concentrations lead to an infection risk of 0.09 to 1.2% (Table 5). At the recreational sites, the low average parasite concentrations give rise to an estimated infection risk of 0 to 0.0009% for *Cryptosporidium* and 0 to 0.009% for *Giardia*, whereas the maximum concentrations result in an infection risk of 0 to 0.006% for *Cryptosporidium* and 0 to 0.06% for *Giardia* (Table 5). Figure 2 displays estimated infection risks for ingested volumes of 0 to 100 ml.

DISCUSSION

Water quality. The water in the Amsterdam canals Prinsengracht and Herengracht is fecally contaminated. Testing for compliance with the stringent standards outlined in revised European Bathing Water Directive 2006/7/EC demonstrated that water quality was poor and unsuitable for safe swimming. The water contained high numbers of fecal indicators, and the presence of the waterborne pathogens *Salmonella*, *Campylobacter*, *Cryptosporidium*, *Giardia*, rotavirus, norovirus, enterovirus, and reovirus was confirmed in several samples. International literature does not provide much information on the microbiological quality of canal water in other Western cities. In the 1980s, *Aeromonas sobria* was isolated from canal water in London (42). The water isolates could be related to fecal isolates from children in a children's hospital. Some other papers report the presence of pathogens in city canals in less developed countries. Large numbers of *Enterobacteriaceae*, including a wide range of *Salmonella* serotypes, were found in the city canals in Jakarta (25). In a Thai study, *Campylobacter* was detected in the canals of the Bangkok Metropolitan Area (22), whereas *Giardia* cysts were found in Mexico City canals (32). However, in these parts of the world, city canals serve different purposes than canals in cities in developed countries,

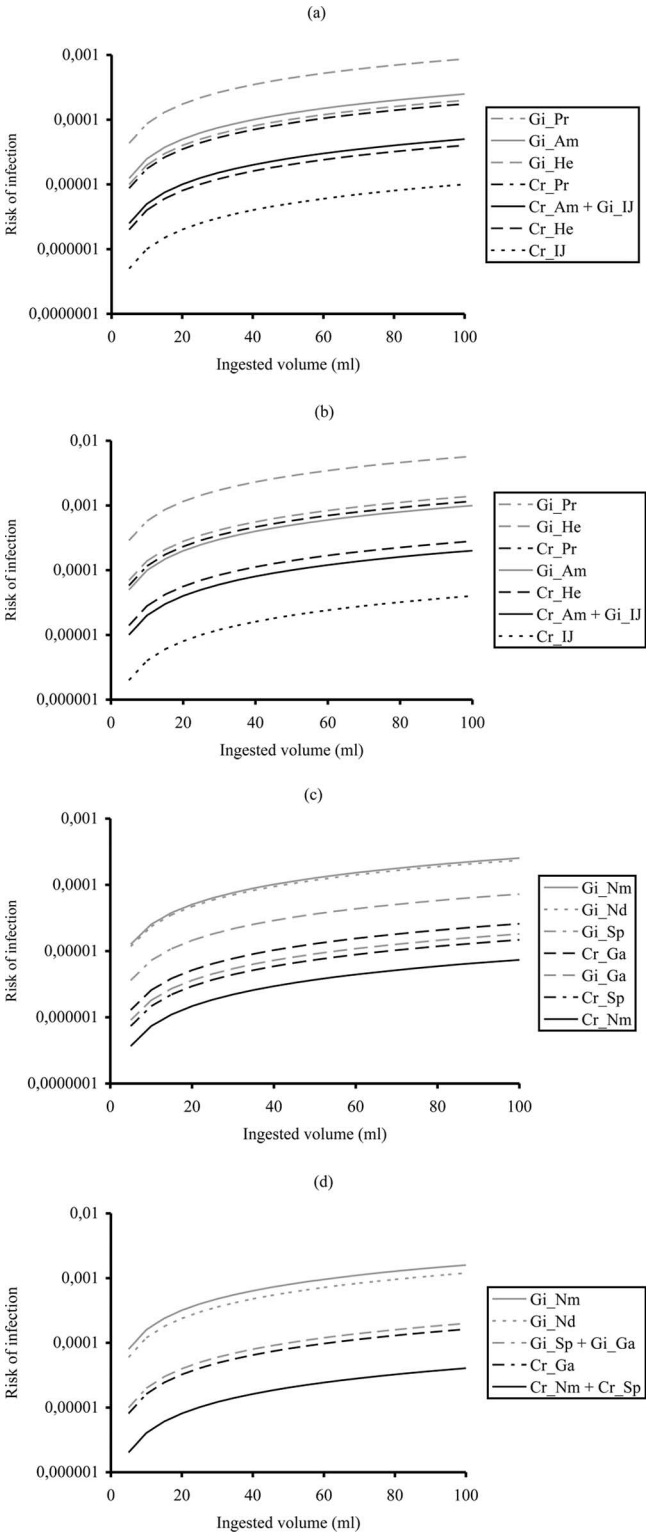

FIG. 2. The risk of infection with *Cryptosporidium* (Cr) and *Giardia* (Gi) in surface water in Amsterdam for mean (a) and maximum (b) concentrations detected at nonrecreational sites (Pr, Prinsengracht; He, Herengracht; Am, Amstel; IJ, IJmeer) and mean (c) and maximum (d) concentrations detected at recreational sites (Nd, Nieuwe Diep; Sp, Sloterplas; Nm, Nieuwe Meer; Ab, Amsterdamse Bos; Ga, Gaasperplas).

such as washing, drinking, and cooking, and are subject to other contamination sources, like the disposal of raw waste by the part of the population that is not connected to the sewer systems. The Amsterdam canals are not designated for recreation, but exposure does occur through professional, accidental, and purposeful contact. In the cases of the last two, information should be provided to the public to prevent exposure, whereas in the case of the first, advice should be given on the protection of, e.g., professional divers (49).

Contamination sources. The previously reported relation between heavy rainfall and increased numbers of fecal indicators in surface water (24, 45) was confirmed for the Amsterdam canals. High fecal indicator concentrations, the detection of elevated numbers of *Cryptosporidium* and *Giardia* (oo)cysts, and the occurrence of *Salmonella* and culturable enteroviruses appeared to be related to rainfall events of high intensity. During these events, dirt from the streets is washed into the canals, sewage systems may overflow, and discharged water from nearby polders containing runoff from agricultural land is transported into the canals. High fecal indicator counts and rainfall were less obviously related in the recreational lakes in Amsterdam. Only extreme rainfall intensity caused peaks in indicator levels at all recreational sites, suggesting that the lakes are subject to sewage overflow and surface runoff to a lesser extent than the nonrecreational sites.

The assay applied for the detection of culturable entero- and reoviruses detects only human viruses, indicating that the culturable enteric viruses in the canals and the Amstel were most likely of human origin (37). Enteroviruses (29) and reoviruses (31) have been demonstrated to be the cause of meningitis in humans, and therefore, their presence in the Amsterdam canals may pose a health threat to people who are exposed to this water. However, because of limited quantitative virus data and the unavailability of dose-response parameters for reoviruses, no attempts were made to estimate the risk of infection with these viruses. The molecular detection of pathogenic rota- and noroviruses indicates the possible presence of infectious virus particles. Moreover, it has been shown that at low levels of norovirus PCR-detectable units, both infection and illness may be established in human volunteers (35).

The isolated *Salmonella* species can cause human GE, and their presence therefore poses a health risk. *Salmonella* serovars Virchow and Newport, which were isolated from the Amstel, have been isolated from both animal and human samples in The Netherlands (39, 40). Increased antibiotic resistance of these *Salmonella* types, which was observed in France (17) and the United States (38), has not been observed in The Netherlands to date (39, 40). In The Netherlands, *Salmonella* serovar Typhimurium phagetype 690, isolated from the Herengracht canal, has been found mainly in doves, but also in humans (W. van Pelt, RIVM, personal communication).

The detection of *Campylobacter* was frequent at all nonrecreational sampling sites and was not related to heavy rainfall events, suggesting that sources such as sewage overflow and runoff from agricultural land and streets play a less profound role. Large numbers of ducks and gulls are regularly observed in the Amsterdam canals. Considering the frequent isolation of *Campylobacter* from various birds (15, 34, 41, 52, 60), including ducks and gulls, the direct input of bird feces may be an important source of *Campylobacter* contamination of the canal

water. Bird types may be zoonotic and pose a potential risk for public health.

The levels of fecal indicator parameters in the canal with houseboats (Prinsengracht) and the canal without houseboats (Herengracht) were almost equal and did not suggest that houseboats contributed to the fecal contamination of canals to a larger extent than other sources did. However, *Cryptosporidium* and especially *Giardia* numbers were much higher in the Prinsengracht canal than in the Herengracht canal, suggesting that wastewater from houseboats may have been a source of these parasites. Moreover, the Prinsengracht canal was the only site for which there was no correlation between parasite concentrations and rainfall, suggesting that sources other than sewage overflow caused contamination of the water. Data on the prevalence of *Giardia* infections among people who live on houseboats are not available. Typing could have provided more information on the origin of the isolated *Giardia* cysts but was not included in the original assignment. Retrospective genotyping of (oo)cysts present in the stored remainder of the concentrated samples failed due to low (oo)cyst numbers in the concentrates and the limited sensitivity of the available molecular methods.

Risk assessment. Our data indicate that there is a health risk for occupational divers and people who are accidentally exposed to pathogens in the water of the Amsterdam canals. For divers swallowing maximum volumes of approximately 6 ml canal water (49), the estimated infection risk per dive is generally low (0.0002 to 0.001% for *Cryptosporidium*; 0.04 to 0.06% for *Giardia*). Exposure to incidental peak concentrations that were detected in the canal Prinsengracht and the river Amstel may, however, result in higher infection risks per dive (0.007% for *Cryptosporidium* and 0.2% for *Giardia*). Most pathogens detected in the canals cause mild illness such as GE; prevention of infection can be achieved by minimizing the ingested volume of water as much as possible. Wearing a full face mask provides more protection than an ordinary diving mask (49). People who are accidentally exposed to the canal water and presumably swallow more water than divers, but at most the same volume as nonadult swimmers (37 ml) (23), are particularly at risk for an infection with *Giardia*. When cyst concentrations peak, like in the Prinsengracht canal and the Amstel, the infection risk is 1.0 to 1.2% per exposure event, indicating that approximately 1 in 100 exposed persons may become infected.

The recreational lakes in Amsterdam contained viable *Cryptosporidium* and *Giardia* (oo)cysts, despite their compliance with European bathing water legislation. The concentrations were lower than those in canals, and consequently, the estimated risks of infection per exposure event were generally 10- to 1,000-fold lower. The risk estimates were consistent with results reported by Coupe et al. (19), who estimated the risk of infection with *Cryptosporidium* and *Giardia* associated with swimming in surface water near Paris, France. They reported infection risks below 0.01% when (oo)cyst concentrations were less than 2 per 10 liters. These concentrations were observed both in Paris and in Amsterdam recreational lakes. (Oo)cyst concentrations of 2 or more per 10 liters, found in canal water in Amsterdam and river water near Paris, resulted in risks of infection of 0.01% or more.

From a prospective population-based cohort study, the GE incidence for the general population in The Netherlands was estimated to be 283 per 1,000 person-years, indicating an average annual GE risk of 28% (21). The case control study that was nested in this cohort study yielded estimated average risks of GE due to *Giardia* and *Cryptosporidium* infections of 1.4% and 0.6%, respectively. For *Giardia*, infection risks due to exposure to canal water were estimated in our study to be approximately 2 to 50 times lower. However, when cyst concentrations peak, the infection risk per exposure event is in the same order of magnitude as reported by de Wit et al. (21), namely 1.0 to 1.2%. For *Cryptosporidium*, the estimated infection risks due to canal water exposure are 15 to 10,000 times below the baseline level of 0.6%. For the recreational lakes, the estimated risks of infection per exposure event are 10 to 1,000 times lower than those estimated for canal water exposure. Although the frequency of exposure to canal water and recreational lakes in Amsterdam is unknown, the number of GE cases caused by *Giardia* or *Cryptosporidium* as a result of contact with these waters will most likely not exceed the baseline level of *Giardia* or *Cryptosporidium* GE cases in the general Dutch population and will go unnoticed.

The infection risks we report here may be overestimated since a fraction of the (oo)cysts that were considered viable based on the outcome of the applied viability tests may not be infectious. It has been demonstrated that the results of viability assays do not always correlate with the outcome of in vivo (43) and in vitro infectivity assays (16). Moreover, parasite genotypes could not be confirmed and a fraction of the (oo)cysts may belong to species other than those infectious to humans. It should also be noted that *Cryptosporidium* and *Giardia* analyses in the recreational lakes obtained many zero counts, resulting in increased uncertainties in infection risk estimates.

The results of this study demonstrate the presence of waterborne pathogens in surface water in Amsterdam and show that both occupational and accidental exposure to water in the Amsterdam canals may pose a health risk. Although the Amsterdam canals are unique, their microbiological status may not be very different from canals in other developed countries, and therefore, the data presented here may be of use for health care workers in other cities. The presence of *Cryptosporidium* and *Giardia* in recreational lakes may pose a possible health risk for bathers, despite fecal indicator parameters indicating safe swimming.

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