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Occurrence of *Cryptosporidium* Oocysts and *Giardia* Cysts in Sewage in Norway†

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Samples of sewage influent from 40 sewage treatment works (STW) throughout Norway were examined for *Cryptosporidium* oocysts and *Giardia duodenalis* cysts. Both parasites were detected frequently (80% of STW were *Cryptosporidium* positive; 93% of STW were *Giardia* positive) and at maximum concentrations of >20,000 parasites/liter. The data suggest giardiasis is more widespread, and/or occurs with greater infection intensity, than cryptosporidiosis in Norway. STW serving higher person equivalents were more likely to be positive and had higher parasite concentrations. Parasite concentrations were used to estimate the proportion of contributing populations that could be clinically infected. For *Cryptosporidium*, the highest estimates were up to 5 per 100,000 individuals for two populations in eastern Norway. For *Giardia*, the highest estimate was 40 infected per 100,000 persons (approximately five times the usual national annual average) contributing to an STW in western Norway. As this population experienced a large waterborne giardiasis outbreak 6 months after sampling, it can be speculated that regular challenge with *Giardia* may occur here. Most *Giardia* isolates in sewage influent were assemblage A, although some assemblage B isolates were detected. There was substantial heterogeneity, but most samples contained isolates similar to genotype A3. Removal efficiencies at two STW with secondary treatment processes were estimated to be approximately 50% for *Cryptosporidium* and >80% for *Giardia*. An STW with minimal treatment had negligible removal of both parasites. Many STW in Norway have minimal treatment and discharge effluent into rivers and lakes, thus, risk of contamination of water courses by *Cryptosporidium* and *Giardia* is considerable.

Analysis of sewage influent for *Cryptosporidium* oocysts and *Giardia duodenalis* cysts has been used in a variety of studies to further elucidate aspects of the epidemiology, both conventional and molecular, of these parasites in particular populations or geographic regions (1, 20). Additionally, such analyses can be used as an indirect method of assessing the occurrence of these infections in human populations (12, 17, 18). This is particularly useful in situations where it is believed that the occurrence of these infections is underestimated.

Investigation of the epidemiology of cryptosporidiosis and giardiasis in human populations in Norway is hampered by lack of submission of fecal samples to diagnostic laboratories. This may be due to low infection rates but is more probably due to lack of awareness of these infections among medical personnel. In the absence of such specimens, investigation of sewage influent for these parasites can provide a useful approach for collecting data on the extent of infection in different regions. However, the method for analyzing such samples must be chosen carefully; particulate debris, fats, and other contaminants from a range of sources mean that standard water analysis procedures are inappropriate. Minimizing sample manipulation procedures has previously been shown to be the most efficient approach (16), but limits of detection must also be considered.

If molecular tools are used in addition to detection and enumeration, information on the species/genotypes occurring in the population may give further insight into the epidemiology of the infections.

Measurements of concentrations of parasites in sewage influent over time may provide information on temporal variation in the occurrence of infections, but as flow rate also varies according to precipitation and industrial contributions, such data must be treated cautiously.

Sewage effluent may be a source of contamination of the environment, which may be of public health significance, particularly if sewage is discharged into water that is subsequently used for drinking, recreation, or agricultural purposes. Thus, analysis of sewage effluent and estimation of the removal efficiencies of sewage treatment works (STW) incorporating different treatment regimens may provide useful information on potential contamination of water supplies.

Here, the various aspects listed above were used to accrue data on the occurrence, epidemiology, and potential for transmission of *Cryptosporidium* and *Giardia* infections in Norway.

MATERIALS AND METHODS

Sample collection. Between March and July 2004, single samples of sewage influent (50 to 500 ml) were collected from at least two sewage treatment works (STW) in each of the 19 counties of Norway (for 2 counties, Nord-Trøndelag and Sør-Trøndelag, three STW were sampled). For every county, where possible, one sample came from a large STW serving a mainly urban population, and one sample came from a smaller STW serving a rural population.

In total, 40 different STW were sampled, and information about each STW was collected retrospectively by questionnaire. Completed questionnaires were re-

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TABLE 1. Characteristics of some of the STW included in the survey

Measurement	Result	Median; range
No. of STW sampled[e]	40	
No. of STW for which information was obtained	34[a]	
Mean no. of PE served by STW (n = 34)	38,900	9,650; 50–440,300
No. of STW (% of 34) serving 50–900 PE (group 1); (mean PE)	9 (26.5); (320)	280; 50–600
No. of STW (% of 34) serving 1,000–9,900 PE (group 2); (mean PE)	8 (23.5); (3,850)	3,200; 1,300–9,300
No. of STW (% of 34) serving over 10,000 PE (group 3); (mean PE)	17 (50); (75,760)	45,000; 10,000–440300[b]
Mean daily influent vol, in m^3 (n = 28)[c]	28,600	5,000; 40–300,000
Mean daily influent vol, in m^3, for group 1 (n = 6[c])	100	75; 40–200
Mean daily influent vol, in m^3, for group 2 (n = 6[c])	1,500	1,000; 200–3,300
Mean daily influent vol, in m^3, for group 3 (n = 16[c])	49,500	24,600; 2,000–300,000
Total (% of 32[c]) no. of STW with significant input from animal sources (e.g., abattoirs, veterinary establishments, etc.) in group 1 (n = 8[c]), group 2 (n = 7[c]), and group 3 (n = 17)	9 (28) (group 1, 0; group 2, 2; group 3, 7)	
Treatment		
Total no. of STW (% of 32[c]) in group 1 (n = 8[c]), group 2 (n = 7[c]), and group 3 (n = 17) with only primary mechanical treatment (straining/sand/fat removal/primary settlement)	13 (41) (group 1, 4; group 2, 3; group 3, 6)	
Total no. of STW (% of 32[c]) in group 1 (n = 8[c]), group 2 (n = 7[c]), and group 3 (n = 17) with primary mechanical treatment and chemical treatment followed by flocculation/settlement	17 (53) (group 1, 4; group 2, 4; group 3, 9)	
Total no. of STW (% of 32[c]) in group 1 (n = 8[c]), group 2 (n = 7[c]), and group 3 (n = 17) with biological treatments (e.g., activated sludge)	15 (48) (group 1, 4; group 2, 5; group 3, 7)	
Total no. of STW (% of 31[c]) in group 1 (n = 8[c]), group 2 (n = 7[c]), and group 3 (n = 17) with tertiary treatments (filtration, nitrogen removal, disinfection)	3 (10) (group 1, 0; group 2, 0; group 3, 3[d])	
Sewage discharge		
No. of STW discharging by location (n = 28[c])	Sea, 15; lake, 6; river, 7	

[a] Questionnaires were returned from 32 STW, although many omitted to answer, or were unable to answer, various questions. Information on two STW was obtained via the Internet and other sources.
[b] Only two STW served a PE greater than 100,000.
[c] Relevant information was not available from all questionnaires.
[d] Two STW had nitrogen removal, one had UV treatment of effluent.
[e] Sample collection was intended to be completed in March 2004. Twenty samples were collected in March, nine in April, four in May, two in June, and two in July 2004. For those STW which were sampled over time, results from a sample within this period were selected at random for comparison purposes, apart from one STW which had terminated sample collection. For this STW, results from a sample from the previous October were included in the comparisons.

turned for 31 STW; for 2 further STW some of the information was obtained from the Internet and other sources. For data analysis, the STW were divided into three groups, according to STW size as defined by person equivalents (PE) served (Table 1).

Additionally, samples of both influent and effluent were collected simultaneously approximately every 2 weeks for 18 months at two of the STW or for 8 months at one of the STW.

Sample analysis for parasite occurrence. As manipulation steps in sample analysis result in parasite losses, examination of five replicates of 50-μl native samples applied directly (no manipulation steps in processing) to welled microscope slides (Dynal Spot-On slides; Dynal Biotech, Oslo, Norway) was compared to replicates of larger volumes (five replicates of 2 ml or three replicates of 20 ml). These larger subsamples were concentrated and purified using three manipulation steps, i.e., (i) washing in membrane buffer (22); (ii) concentration by centrifugation; and (iii) modified immunomagnetic separation (IMS; GC Combo; Dynal Biotech, Oslo, Norway) using a previously described method (13, 15). A 50-μl purified subsample resulted from each 2-ml or 20-ml sample, all of which was applied to a welled microscope slide. After air drying and methanol fixing, all samples were stained with a monoclonal antibody cocktail against *Giardia* cysts and *Cryptosporidium* oocysts (Aqua-Glo; Waterborne Inc., New Orleans, La.) as well as 4′6 diamidino-2-phenyl indole (DAPI) and were examined by fluorescence microscopy. Parasites in each slide well were enumerated, and the mean result of the replicates was used to extrapolate to concentrations of parasites per liter.

Viability assessment. For five samples of sewage influent from two STW, the parasites were isolated by IMS. Their viability was then estimated in suspension by a modification (14) of an earlier method (3) based on morphological criteria and inclusion/exclusion of the vital dyes propidium iodide and DAPI.

DNA isolation. For selected samples which contained relatively high concentrations of nucleated (oo)cysts, the remaining sewage influent (which had not been examined) was concentrated by centrifugation. The parasites were isolated by IMS (GC Combo) following the manufacturer's instructions, but with multiple washes of the beads before dissociation of the beads and the parasites and resuspension of the isolated parasites in Tris-EDTA buffer rather than drying to slides. The suspensions were held for 1 h at 100°C, and DNA was isolated using a QIAamp DNA mini kit (QIAGEN GmbH, Germany).

PCR, electrophoresis, purification of PCR product, and sequencing. Three different published primer sets were used for investigating *Giardia* genotypes, amplifying sequences of the β-giardin gene (2), glutamate dehydrogenase (*gdh*) gene (11), and small subunit (SSU)-rRNA gene (7). A single primer set, amplifying a sequence of the *Cryptosporidium* oocyst wall protein (COWP) gene, was used for investigating *Cryptosporidium* (19). For all genes the following PCR mixture was used: 10 pmol of each primer, 0.4 μl bovine serum albumin (20 mg/ml), 17.6 μl water, 25 μl HotStartTaqmaster (QIAGEN GmbH, Germany), and 3 μl DNA. For each reaction set, negative and positive controls were included. PCR products were electrophoresed on 1% agarose gels and stained with ethidium bromide.

Following successful PCR, the products were purified (High Pure PCR Product Purification kit; Roche Diagnostics GmbH) and sequenced on both strands (MWG Biotech, Germany). Chromatograms and sequences were examined using Chromas (http://www.technelysium.com.au/chromas.html) and BioEdit (http://www.mbio.ncsu.edu/BioEdit/page2.html). Sequence searches were conducted using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

Statistics. Contingency tables were prepared to compare numbers of positive and negative samples obtained from different analytical sample sizes, with Chi-

TABLE 2. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in influent and effluent samples from Norwegian STW

Sample type	Results for[a]:			
	Cryptosporidium		*Giardia*	
	50-μl native samples	2-ml samples	50-μl native samples	2-ml samples
Influent samples from 40 different STW across Norway				
No. (%) of STW positive	16 (40) ac	32 (80) ad	22 (55) bc	37 (93) bd
Range of oocyst/cyst numbers detected (extrapolated to per liter)	4,000–24,000	100–1,100	4000–28,000	100–13,600
Mean concn[b] (SD) (extrapolated to per liter)	6,781 ei (5,935)	242 ej (226)	7,595 fi (6,676)	1,903 fj (2,833)
Median concn[b] (extrapolated to per litre)	4,000	200	4,000	800
Effluent samples ($n = 72$) from 3 different STW				
No. (%) of positive samples	29 (40) km	44 (61) kn	32 (44) lm	53 (74) ln
Range of oocyst/cyst numbers detected (extrapolated to per liter)	4,000–36,000	100–44,500	4,000–44,000	100–51,333
Mean concn[b] (SD) (extrapolated to per liter)	10,429 go (10,405)	1,316 gp (6,675)	12,387 ho (10,295)	3,029 hp (7,708)
Median concn[b] (extrapolated to per liter)	4,000	100	8,000	500

[a] Numbers followed by the same lowercase letter have been compared statistically (Fisher's exact probability test or two-sided *t* test, as appropriate). The following list describes the significance obtained. a, $P = 0.0005$; b, $P = 0.0003$; c, $P = 0.26$ (no significant difference); d, $P = 0.19$ (no significant difference); e, $P < 0.02$; f, $P < 0.03$; g, $P < 0.00003$; h, $P < 0.00001$; i, $P > 0.05$ (no significant difference); j, $P < 0.002$; k, $P = 0.0193$; l, $P = 0.0006$; m, $P = 0.736$ (no significant difference); n, $P = 0.155$ (no significant difference); o, $P > 0.05$ (no significant difference); p, $P > 0.05$ (no significant difference).

[b] Positive samples only.

squared and Fisher's exact tests used for the analyses. Mean concentrations were compared by two-sided *t* tests.

RESULTS

Comparison of 50-μl, 2-ml, and 20-ml subsamples. In all cases where parasites were detected, extrapolation to number of (oo)cysts per liter gave significantly higher results from 50-μl native samples than from 2-ml equivalent samples (Table 2; for influent samples, $P < 0.02$ for *Cryptosporidium* and $P < 0.03$ for *Giardia*; for effluent samples, $P < 0.00003$ for *Cryptosporidium* and $P < 0.00001$ for *Giardia*). The results for the latter were higher than those from 20-ml equivalent samples (only influent samples were analyzed; data not shown). However, relationships between extrapolated results from the three different sample sizes varied considerably. Sometimes the extrapolated results from 50-μl native samples would be approximately the same as those from 2-ml equivalent samples but often could be many times higher, varying from twice as high to up to 50 times as high, with particularly variable and large differences in sewage influent. A similar situation was seen with 20-ml equivalent samples but with even more pronounced variability, hence only results from the two smaller sample volumes were used.

For both parasites, 2-ml equivalent samples yielded significantly more positive samples than the 50-μl native samples ($P < 0.0001$).

Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in sewage influent. Although for both 50-μl native samples and 2-ml equivalent samples more *Giardia*-positive samples than *Cryptosporidium*-positive samples were detected, the differences were not significant (Table 2).

Both parasites were detected in the same sample from 10 STW (25%) using 50-μl native samples and in 28 STW (70%) using 2-ml samples. With 2-ml equivalent samples, either *Cryptosporidium* or *Giardia* was detected in samples from all STW investigated (i.e., there was no STW in which at least one parasite was not detected). Using 2-ml samples, larger STW (group 3) did not have significantly more or fewer positive samples than smaller STW (group 1); however, for 50-μl samples, more positive samples were obtained from bigger STW for both *Cryptosporidium* and *Giardia*.

The mean concentration of *Giardia* cysts was significantly higher than the mean concentration of *Cryptosporidium* oocysts in 2-ml samples (Table 2; two-sided *t* test, $P < 0.002$) but not in 50-μl samples. Only 2-ml sample results were used for comparisons of parasite concentrations with size of treatment plant, as so many 50-μl samples were negative. The only significant difference between STW groups was between *Giardia* cyst concentrations recorded in the largest STW (group 3; mean of 2,400 cysts per liter) and those recorded in the smallest STW (group 1; mean of 275 cysts per liter; two-sided *t* test, $P = 0.031$).

For the three STW at which samples were taken regularly over a period of 8 to 18 months, concentrations for both parasites fluctuated considerably between sampling occasions. For example, extrapolated concentrations of *Giardia* cysts ranged from 100 to over 20,000 cysts per liter at the same STW, with less than 6 weeks between sampling occasions. No pattern of seasonality or any other factor could be detected for individual STW, and there was no consistent pattern in fluctuations among the three STW.

Estimation of human contribution to parasites detected in sewage influent. No significant association between parasite occurrence and STW which reported input from animal sources was detected, and parasite concentrations did not differ significantly between those with and without a record of animal input.

Using STW data (summarized in Table 1) and concentrations of parasites detected in influent (from 2-ml samples), the daily oocyst/cyst load on each STW was estimated. If the (oo)cyst excretion rate associated with a heavy (and therefore symptomatic) infection can be assumed to be approximately 10^{10} per day for both parasites, then the number of symptomatic persons associated with a particular STW can be estimated (Table 3). Thus, the highest number of individuals with symptomatic cryptosporidiosis served by a particular STW was es-

TABLE 3. Extrapolation from data summarized in Tables 1 and 2 (2-ml data) and used to estimate symptomatic infection numbers in communities served by different STW at sampling date

STW group	Mean estimated no. of infected persons per STW[a]		Range of estimated no. of infected persons per STW[a]		Mean estimated % of PE served with symptomatic infections per STW	
	Cryptosporidium	*Giardia*	*Cryptosporidium*	*Giardia*	*Cryptosporidium*	*Giardia*
All STW ($n = 23$ for *Cryptosporidium*, $n = 27$ for *Giardia*)	1	8	<1–6	<1–132	0.001[b]	0.001[c]
STW in groups 1 and 2 combined ($n = 9$ for *Cryptosporidium*, $n = 11$ for *Giardia*)	<1	<1	<1	<1–1	<0.001	0.006
STW in group 3 ($n = 14$ for *Cryptosporidium*, $n = 16$ for *Giardia*)	1	13	<1–6	<1–132	0.002	0.012

[a] Rounded to the nearest integer.
[b] Two STW (both in group 3) estimated to serve populations where more than 5 in 100,000 persons (percentage of PE served, >0.005) have symptomatic *Cryptosporidium* infection.
[c] Six STW (one in group 2 and five in group 3) estimated to serve populations where more than 2 in 10,000 persons (percentage of PE served, >0.02) have symptomatic *Giardia* infection. Of these, one STW estimated to serve a population where more than 4 in 10,000 persons (percentage of PE served, >0.04) have symptomatic *Giardia* infection.

timated to be 6, and the highest number of individuals with symptomatic giardiasis served by a particular STW was estimated to be 132 (Table 3). Further extrapolation from the data summarized in Table 1 enables this number to be described as a percentage of the PE served by a particular STW or as the number of cases per 100,000 persons (Table 3). Thus, two STW served populations with an estimate of more than 5 symptomatic cases of cryptosporidiosis per 100,000 persons, whereas six STW served populations with an estimate of more than 20 cases of symptomatic giardiasis per 100,000 persons, one of which served a population with an estimate of more than 40 cases of symptomatic giardiasis per 100,000 persons. The two STW with the highest estimates of cryptosporidiosis cases were both large (serving PE of 120,000 and 85,000), and both were in eastern Norway (on opposite sides of Oslo Fjord). Both listed prisons and hospitals as being among their contributors; in addition, one reported an asylum center and the other an abattoir. Five of the six STW with an estimate of >20 giardiasis cases per 100,000 persons were in group 3 (serving over 10,000 PE), and the was other in group 2 (serving a population of 4,200 PE). Four were in eastern Norway, one in northern Norway, and one in western Norway. Associated contributors which may have resulted in the relatively elevated *Giardia* cyst concentrations at some of these STW included hospitals, prisons, slaughterhouses, and Norway's main international airport. The STW with the largest number of estimated giardiasis cases was located in Bergen.

Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in sewage effluent and estimated removal efficiencies. Seventy-two effluent samples were examined during the study from three different STW (32 samples from STW B, 15 from STW V, and 25 from STW F). The combined results from the three STW (Table 2) demonstrate that although for both 50-μl native samples and 2-ml equivalent samples there were more *Giardia*-positive samples than *Cryptosporidium*-positive samples, the difference was not significant. Also, the concentration of *Giardia* cysts was not significantly higher than the concentration of *Cryptosporidium* oocysts for either 50-μl or 2-ml effluent samples (Table 2). Both parasites were detected in 16 (22%) 50-μl samples and in 38 (53%) 2-ml samples.

Mean removal efficiency (removal from liquid phase into solid phase) of the STW for each parasite was estimated using data from 2-ml influent and effluent samples collected on the same day (Table 4) and was found to vary from 0 to 50% for *Cryptosporidium* and 0 to 95% for *Giardia*, depending on the STW. As the theoretical detection limit for this method with five replicates (assuming 100% efficiency of method) can be extrapolated to 100 oocysts/cysts per liter, and as no oocysts/cysts are detected in some cases, it is not possible to determine how far below the detection limit the concentration may be, because removal efficiency was only estimated when parasites were detected in both influent and effluent samples. On some occasions more parasites were detected in the effluent than the influent (three for *Cryptosporidium* in STW B, six for *Cryptosporidium* in STW F, and one for *Giardia* in STW V), but these data were not excluded from the estimations.

Viability assessment. Viability assessments were conducted on five influent samples from two STW. *Giardia* cyst viability

TABLE 4. Estimated removal efficiencies for *Cryptosporidium* oocysts and *Giardia* cysts at three STW

Parameter	Result for STW:		
	B	F	V
No. of influent and effluent samples collected on the same day	30[a]	25[a]	15[b]
No. of influent samples containing *Cryptosporidium* oocysts	27	18	15
No. of effluent samples containing *Cryptosporidium* oocysts	13	20	10
No. of sample pairs to estimate *Cryptosporidium* removal efficiency	13	16	10
Extrapolated mean concn of *Cryptosporidium* (oocysts/liter) in influent	485	3,145	770
Extrapolated mean concn of *Cryptosporidium* (oocysts/liter) in effluent	230	3,300	370
No. of influent samples containing *Giardia* cysts	30	25	15
No. of effluent samples containing *Giardia* cysts	16	25	11
No. of sample pairs to estimate *Giardia* removal efficiency	16	25	11
Extrapolated mean concn of *Giardia* (cysts/liter) in influent	9,520	5,620	3,420
Extrapolated mean concn of *Giardia* (cysts/liter) in effluent	506	5,880	480
Estimated % removal efficiency of *Cryptosporidium* oocysts by STW	50	0	50
Estimated % removal efficiency of *Giardia* cysts by STW	95	0	85

[a] Samples were collected over an 18-month period.
[b] Samples were collected over an 8-month period.

was considered to range between 11% and 30% in different samples (assessment of between 6 and 28 cysts), and *Cryptosporidium* oocyst viability was considered to range between 15% and 50% in different samples (assessment of between 6 and 16 oocysts).

Genotyping/sequence analysis. PCR amplification of COWP gene sequences was unsuccessful for samples containing *Cryptosporidium* oocysts. However, successful PCR was conducted on *Giardia* gene sequences on isolates from 30 influent samples from 20 different STW and 4 effluent samples from 2 different STW (see the table in the supplemental material). For *Giardia* isolates from 4 influent samples and 1 effluent sample, all gene sequences were amplified, but for most sample isolates either one (12 samples) or two (17 samples) genes were investigated (see the table in the supplemental material). Thirty-two samples (89%) were considered to contain *Giardia* cysts from assemblage A, the majority being most similar to genotypes A3 (17 samples) and A2 (10 samples) (see the table in the supplemental material). Evidence of *Giardia* from assemblage B (genotype B3) was found in three influent samples from three different STW (see the table in the supplemental material).

Although different genotypes would be expected in sewage samples, in general the electropherograms were clear with well-defined peaks, indicative of a predominant genotype in each sample. However, use of different primer sets on the same sample did not always produce the same result (see the table in the supplemental material). In three samples in which *Giardia* cysts were considered to be of genotype A3 from the β-giardin gene sequence analysis, results from the *gdh* gene sequence analysis indicated that the sample contained *Giardia* cysts of, or most similar to, genotype A2. In another sample, which was considered to be of assemblage A from the SSU-rRNA gene and A2 from the β-giardin gene, results from the *gdh* gene indicated that the sample contained *Giardia* cysts most similar to genotype B3. A further sample, in which sequence analysis from the β-giardin gene indicated that it contained *Giardia* cysts of genotype B3, was considered to contain *Giardia* from assemblage A from the SSU-rRNA gene.

DISCUSSION

Comparison of analytical methods demonstrated that considerable parasite losses can occur from sewage samples during manipulation steps, as has previously been noted (16). For accurate estimation of parasite concentrations, sample manipulation steps should be minimized. However, when manipulation steps are reduced by using 50-μl native samples, the theoretical limit of detection is so high (4,000 [oo]cysts per liter for five replicate 50-μl native samples) that many samples with lower parasite concentrations are not identified. When using 2-ml samples, the greater volume equivalent examined more than compensates for losses due to the manipulation steps. The theoretical limit of detection (100 [oo]cysts per liter for five replicate 2-ml equivalent samples) is sufficiently low that most positive samples are detected. Therefore, 50-μl and 2-ml equivalent results provide different pieces of information which are both of interest.

Irrespective of analytical method, the sewage influent results demonstrate that *Giardia* and *Cryptosporidium* infections are widespread throughout Norway, with giardiasis being more widespread and/or occurring with greater infection intensity than cryptosporidiosis. STW serving higher PE had higher parasite concentrations and were more likely to be positive than smaller STW; in some instances, these differences were statistically significant. Clearly, the more PE served, the greater the probability that the population will include an infected individual.

In Norway, giardiasis is a reportable infection: 300 to 400 cases are usually recorded annually (8, 9, 10) (approximately 8.5 cases per 100,000 persons), most of whom have allegedly acquired their infections abroad. In 2004, over 1,500 cases of giardiasis were reported (8, 9) due to a waterborne outbreak in Bergen (15, 21), which occurred after the present STW sampling had been completed.

Cryptosporidiosis is not a reportable infection in Norway unless associated with diagnosis of AIDS (10). Data from 14 medical diagnostic laboratories indicate that cryptosporidiosis is seldom diagnosed (maximum of 2 diagnoses annually in 5 of the laboratories). The STW data indicate that both infections are more common than the diagnostic data suggest; enhanced detection of *Cryptosporidium* infections during the Bergen giardiasis outbreak (13) also indicated that this infection is considerably underdiagnosed in Norway.

Extrapolation from the analytical data, combined with STW technical data, enabled estimation of the numbers of individuals infected, assuming that oocysts/cysts in the sewage originated from relatively heavy, and therefore symptomatic, infections. Obviously (oo)cysts in sewage influent will not only be derived from symptomatic persons but also from asymptomatic individuals excreting lower numbers of oocysts/cysts; this is probably particularly so for *Giardia*, which is well recognized for causing chronic infections. Nevertheless, for comparative purposes, it is simpler to assume that all infections are of similar symptomatic intensity.

While the two STW serving the populations with the estimated highest relative number of *Cryptosporidium* infections were located in the same region of Norway, they are sufficiently separated that they cannot be associated. Neither had particular characteristics which would suggest the populations served should demonstrate a greater predisposition to cryptosporidiosis, and although some contributors may be of higher infection risk, other STW with lower *Cryptosporidium* concentrations reported similar contributing populations.

The STW serving the population with the estimated highest relative number of giardiasis infections (approximately five times the number estimated from reports to the Norwegian Institute of Public Health) was in Bergen, where an extensive outbreak of waterborne giardiasis occurred (15, 21) within 6 months of sampling.

Giardia cysts and *Cryptosporidium* oocysts entering STW may either be partitioned into the solid phase or discharged in the effluent. If the discharged parasites have remained infectious, further transmission may occur. Effluent discharge is dependent upon the options available; at coastal locations discharge is frequently to sea, and inland discharge to a river or lake is usual. Although most STW in this study discharged to the sea, at least 13 discharged to a freshwater body or river, of which at least two are drinking water sources and six are used for recreation. Removal efficiencies (i.e., partitioning of [oo]cysts into the solid phase) at two of the STW investigated, which

both incorporated mechanical and chemical treatment, were relatively high. However, as the estimated parasite loads on these STW were considerable, particularly for *Giardia*, parasite discharge in the effluent could also be considerable. Even if only 0.00001% of parasites discharged were viable, this could still result in a daily discharge of over a million viable *Giardia* cysts. At these two STW, removal of *Cryptosporidium* oocysts was lower, presumably because of their smaller size, though biophysical/biochemical differences may also influence removal. At the third STW, for which a primary coarse screening was the only treatment process, no removal of either parasite could be ascertained, and it can be assumed that other STW with minimal treatment also have low removal efficiencies. Most research dealing with parasite removal at STW have investigated STW with different secondary or tertiary treatments and have reported removal efficiencies of around 90% for *Giardia* cysts (1, 5, 23), with that for *Cryptosporidium* (when assessed) usually lower and more variable. However, other studies which have examined STW with only primary treatments or examined each treatment individually generally conclude that removal efficiencies of primary steps are low (4, 16). Many STW in Norway use primary treatment processes only, and it should be assumed that at such STW removal of these parasites from the sewage influent is minimal.

For some samples (at all three STW), the number of parasites detected in the effluent was greater than in the influent sample collected on the same day. This may be an artifact of higher method recovery efficiencies with the cleaner effluent samples or a reflection of temporal fluctuations in parasite concentrations, the lack of "pairing" of the samples, the uneven distribution of parasites in the sample matrices, or a combination of these factors. Similarly, removal efficiency estimates should also be treated with caution.

Failure to obtain positive PCR results from wastewater samples containing *Cryptosporidium* oocysts has also been reported by others (1). It is unknown whether this was due to insufficient DNA or inhibition of the PCR.

In our study, *G. duodenalis* genotypes associated only with animal infections were not identified, suggesting that the majority of the cysts in the sewage originated from human infections. Additionally, most STW did not report significant animal waste input into the STW. Assemblage A *Giardia* (genotypes A2 and A3) appeared to be more common and more widespread than those from assemblage B. An Italian study (1) also demonstrated that assemblage A *Giardia* cysts occurred more frequently than assemblage B in sewage samples. In an STW study in Milwaukee (20), *Giardia* cysts isolated from approximately 85% of 131 wastewater samples were assemblage A, which is similar to our results. However, in the Milwaukee study, by sequencing at a single gene, over 96% of the assemblage A isolates were considered to be of identical subgenotype. In our study, greater diversity was detected among the isolates. This might be because the Milwaukee study focused upon a single STW, whereas our study involved samples from various geographically distinct STW. However, in our study, *Giardia* isolates from six different influent samples from the same STW were genotyped at the β-giardin gene and four different sequences were obtained (one assemblage B and three different assemblage A), suggesting greater heterogeneity than in the Milwaukee study.

That five samples in our study gave differing results when examined at different genes is interesting. Although a mixture of different isolates would be expected in sewage, results from any single gene indicated predominance of a particular isolate in a single PCR run, as the electropherograms tended not to demonstrate multiple peaks. One can speculate that perhaps particular PCR conditions may favor particular sequences, or that the random distribution of cysts may result in one isolate predominating on one occasion and another isolate predominating on another. These results suggest that isolate heterogeneity within a sample should not be excluded unless PCR analysis is repeated, either with the same or different primer sets.

Some studies (6) have suggested that assemblage A infections may be less symptomatic than those from assemblage B, and this may partly explain the relative lack of diagnoses in Norway compared with the high numbers of cysts detected in sewage. The giardiasis outbreak in Bergen in 2004 and 2005 was caused by a genotype related to B3 (15); the main STW for Bergen (code HO) was sampled approximately 3 months before the estimated date of the contamination event, which is presumed to have resulted from sewage leakage into the water supply. Although this STW was estimated to serve a community with relatively high numbers of symptomatic cases of giardiasis, *Giardia* cysts of assemblage B were not detected (two genes were studied). It is therefore possible that the population of Bergen has been frequently challenged with *Giardia* infection usually of assemblage A, and hence it was not immune to the genotype in the outbreak, and/or the strain causing the outbreak was particularly virulent.

Contamination of the water supply in the outbreak was associated with heavy precipitation (15, 21); this weather pattern is typical of Bergen, and concentrations of indicator bacteria (*Escherichia coli*) in Bergen's water sources have been previously demonstrated to be elevated to those levels associated with the outbreak or even higher (21). It is therefore tempting to speculate that perhaps similar contamination events may have occurred on previous occasions, resulting in transmission of less virulent genotypes of *G. duodenalis* to the population via the water supply without causing a recognized symptomatic outbreak.

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