Virus contamination from operation and maintenance events in small drinking water distribution systems

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ABSTRACT

We tested the association of common events in drinking water distribution systems with contamination of household tap water with human enteric viruses. Viruses were enumerated by qPCR in the tap water of 14 municipal systems that use non-disinfected groundwater. Ultraviolet disinfection was installed at all active wellheads to reduce virus contributions from groundwater to the distribution systems. As no residual disinfectant was added to the water, any increase in virus levels measured downstream at household taps would be indicative of distribution system intrusions. Utility operators reported events through written questionnaires. Virus outcome measures were related to distribution system events using binomial and gamma regression. Virus concentrations were elevated in the wells, reduced or eliminated by ultraviolet disinfection, and elevated again in distribution systems, showing that viruses were, indeed, directly entering the systems. Pipe installation was significantly associated with higher virus levels, whereas hydrant flushing was significantly associated with lower virus levels. Weak positive associations were observed for water tower maintenance, valve exercising, and cutting open a water main. Coliform bacteria detections from routine monitoring were not associated with viruses. Understanding when distribution systems are most vulnerable to virus contamination, and taking precautionary measures, will ensure delivery of safe drinking water.

Key words | community water system, distribution system, drinking water, virus

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INTRODUCTION

Drinking water distribution systems require a number of operational and maintenance practices and repairs to maintain system integrity and preserve water quality. For example, flushing sections of the distribution system is a common method to eliminate particulate and soft deposits and reduce odor, taste, and turbidity issues (National Research Council 2006) as well as reduce heterotrophic bacteria counts (Lehtola *et al.* 2004). Finished water storage facilities such as water towers are periodically inspected and cleaned, and valves are routinely exercised (i.e. opened and closed) to ensure they are operational. Repairs for water main breaks happen between 75,000 and 240,000 times per year in the USA, based on national data collected between 1993 and 1996 (Kirmeyer *et al.* 1994; Tafuri 2000). Any of these activities may result in loss of either physical or hydraulic integrity to the system leading to pathogen contamination (National Research Council 2006). To what extent the level of risk for pathogen entry differs among the various distribution system practices a drinking water utility must routinely conduct is not well understood.

The importance of hydraulic integrity in preventing pathogen entry into a distribution system is gaining recognition. When a sudden change in pressure occurs, such as from rapid changes in water demand, power failures, rapid closing or opening of a valve, or a pump starting or stopping, a pressure wave can travel through parts of the system,

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causing transient low or negative pressures (Kirmeyer *et al.* 2001). In a simulation study conducted on three different distribution systems, up to 30% of system nodes experienced negative pressure after events such as main breaks or the use of hydrants to suppress fires (Kirmeyer *et al.* 2001). When water pressure inside the pipe is lower than the outside pressure, liquid and soil material surrounding the pipe can enter the system by suction or gravity through openings, such as cracks or non-sealing joints (Karim *et al.* 2003; LeChevallier *et al.* 2003; Gullick *et al.* 2004). Leaks in municipal distribution systems in the USA account for more than 10% of finished water losses, evidence of the extensive occurrence of such intrusion routes (Kirmeyer *et al.* 2001). Water distribution system pipes are often laid near wastewater pipes, which may leak fecal wastes containing pathogens into the nearby soil. Karim *et al.* (2003) analyzed soil and water samples collected next to drinking water pipes from 32 sites from eight utilities in six states: 18 sites (56.2%) were positive by cell culture or reverse transcription – polymerase chain reaction (RT-PCR) for enteroviruses, hepatitis A virus, or norovirus. Other studies also detected bacterial indicators of fecal contamination in soil and water surrounding drinking water pipes, as well as water present in air-valve vaults (Besner *et al.* 2010). During low or negative pressure events, non-potable water can also enter the system through unprotected cross-connections (US Environmental Protection Agency 2001). Guidelines are available to prevent contamination from low or negative pressure events (Boulos *et al.* 2005), although no specific practice in the USA is enforced by regulation. Once contaminants are introduced into the distribution system, they may be bound or entrained in the biofilm that commonly grows on the pipe surface (Storey & Ashbolt 2003), and subsequently released during events such as valve exercising or hydrant flushing.

In the USA, disease outbreaks associated with distribution systems have increased in relative importance in recent years. During 1991–2000, 21% of all reported drinking waterborne outbreaks were caused by distribution system contamination, compared with 12% during 1981–1990 (Craun *et al.* 2006). Between 2003 and 2006, 50 outbreaks were associated with drinking water. Excluding outbreaks due to point-of-use or premise plumbing contaminations, eight of the remaining 24 outbreaks (33%) were associated with community distribution system deficiencies (Liang *et al.* 2006; Yoder *et al.* 2008).

Sporadic illnesses have also been linked with distribution system performance. Nygård *et al.* (2007), in a cohort study of drinking water consumers in Norway, found that households exposed to water downstream from a distribution system main break or maintenance were 1.6 times more likely to report gastrointestinal illness than households that were not hydraulically downstream from the distribution system event. Hunter *et al.* (2005) in a subanalysis of controls from a case-control study determined there was a strong association between diarrheal illnesses in the control subjects and low water pressure at their home faucets. Tinker *et al.* (2009) observed a slight increase in hospital visits for acute gastrointestinal illness (AGI) in districts consuming water with a longer residence time in the municipal distribution system. In a prospective intervention trial, Payment *et al.* (1997) suggested that pathogen intrusions into the distribution system could be responsible for the higher rates of enteric illness observed in the study group drinking tap water, compared with the group drinking finished municipal water bottled at the treatment plant. All these studies provide epidemiological evidence of a link between distribution system events and endemic illness, but they do not directly investigate the 'intermediate link', i.e. the occurrence of the direct causative agent of disease in the water. Only a recent study by Besner *et al.* (2008) observed a correlation between some distribution system operation and maintenance activities and microbial water quality as indicated by coliform bacteria.

In the present study we assessed whether the detection and concentration of human enteric viruses, the etiologic agents of AGI as well as other more severe diseases, in household tap water are associated with the occurrence of common distribution system events.

METHODS

Study communities

Fourteen rural Wisconsin communities relying on non-disinfected groundwater to supply their municipal water systems were studied. Characteristics of the water systems are

reported in Table 1. The basic hydraulics of the water systems were identical; groundwater was pumped directly into the distribution system with excess water stored in one to three elevated towers that gravity-fed the system when the pumps were off.

Experimental design

This study was part of the larger Water and Health Trial for Enteric Risk (WAHTER), an epidemiological study designed to estimate the proportion of AGI attributable to pathogen contamination of groundwater sources versus contamination occurring in distribution systems. The study was designed as a community-randomized trial with cross-over intervention. During the first year, eight communities received ultraviolet (UV) light disinfection (WEDECO, Charlotte, NC, USA) at all active wells supplying the municipal system, while six communities continued to use untreated water. In the second year, UV intervention was switched to the latter group of communities. The minimum UV dose applied was 50 mJ/cm^2. In each community, human enteric viruses were measured once a month by reverse transcription – quantitative polymerase chain reaction (qRT-PCR) in the wells immediately before and after UV disinfection and at household taps in the distribution systems. Because these communities do not chlorinate and UV disinfection does not leave a residual, viruses were reduced or eliminated at the groundwater source, but not downstream in the distribution system. Therefore, any increase in virus concentration at the household taps downstream from UV disinfection at the wellheads represents virus intrusion into the distribution system. To use this rationale to achieve the study objective, only data collected during the UV intervention periods were used in the analysis (i.e. data from eight communities in Year 1, 2006, and six communities in Year 2, 2007).

Distribution system questionnaire

Water utility managers in the study communities completed a structured questionnaire approximately every 4 months from April 2006 to November 2007. They provided information on operation and maintenance practices, construction, accidents, and routine monitoring in their municipal distribution system. Twelve types of events were queried: preventative or emergency short-term chlorination; shutting down a well pump for repair or maintenance; main breaks; replacing existing distribution system pipes; adding pipes to extend the distribution system; cutting open a water main for reasons other than pipe work (e.g., replacing a hydrant); water tower maintenance; valve exercising; hydrant flushing; cross-connections discovered; routine samples positive for total coliforms; routine samples positive for *Escherichia coli*. Ambiguous answers were resolved by phone call or if necessary by meeting with the utility.

Water sampling and virus enumeration

Tap water samples for viruses were collected once a month from six to eight households in each of the study communities during four periods: April–June, 2006; September–November 2006; March–May, 2007; and September–November 2007 (these periods correspond with the epidemiological data objectives of the WAHTER Study, the results of which are reported elsewhere). Households were selected using maps of water mains provided by the utilities with the goal of finding sample locations representative of different regions of the distribution systems. Viruses were concentrated in the field by glass wool filtration (Lambertini *et al*. 2008). Mean sample volume was 877 L ($n = 902$). After sampling, glass wool filters were stored at 4 °C for <36 hours before further processing.

Filters were eluted with 3% beef extract (wt/vol) containing 0.05 M glycine (pH 9.5), and the eluate concentrated to about 2 ml of final concentrated sample volume (FCSV) as described elsewhere (Borchardt *et al*. 2004; Lambertini *et al*. 2008). Viral nucleic acids were extracted from 280 μL of the FCSV using the QIAamp DNA Blood Mini Kit and Buffer AVL (Qiagen, Valencia, CA, USA) to yield a viral nucleic acid suspension of 50 μL. Two-step qRT-PCR was performed to quantify enteroviruses, noroviruses (genogroups I and II), rotavirus, and hepatitis A virus. Extracted RNA (11.18 μL) was mixed with 11.18 μL of nuclease-free water and 0.91 μL (0.007 μg μL^{-1}) of random hexamers (ProMega, Madison, WI, USA). The mixture was heated for 4 min at 95 °C, then supplemented with 41.7 μL RT master mix containing final concentrations of the following components: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol,

Table 1 | Characteristics of the study communities' municipal water systems

City ID	Population[a]	# Wells (depth range, ft)[b,*]	Aquifer[a]	Mean yield (gal/day)[b,c]	# Water services[b]	Length of system (ft)[b]	Pipe material[b,d]	Annual water loss (%)[b]	# valves[b]	# hydrants[b]	# Water towers (material)[b,f]	Treatment[b,g]
1	8,300	4 (309–450)	Sandstone	3,872,000	3,771	335,408	M, P[d]	13	969	568	3 (S)	F, C
2	1,931	2 (260–266)	Sandstone	223,271	1,125	91,364	M, AC	16	347	151	1 (S)	C
3	1,552	2 (125–424)	Sandstone, sand/gravel	236,734	991	76,440	M	UK[e]	247	158	2 (S, C)	F
4	3,307	4 (350–420)	Sandstone	1,499,969	1,368	133,621	M	3	646	196	1 (S)	C
5	2,311	4 (230–481)	Sandstone	482,605	967	103,050	M, AC, P	16	1,055	180	1 (S)	None
6	3,301	2 (410–417)	Limestone, dolomite	364,633	1,637	137,726	M, P	19	643	307	2 (S)	None
7	3,618	4 (61–88)	Sand/gravel	343,800	1,539	185,880	M, P	2	502	279	2 (S)	F, C
9	1,683	2 (345–383)	Sandstone	228,444	709	61,688	M, P	33	187	114	1 (S)	None
10	1,958	2 (80)	Sand/gravel	131,918	923	93,850	M, P	4	240	133	2 (S, C)	None
11	3,500	2 (111–120)	Sand/gravel, sandstone	301,153	1,451	238,950	M, P	2	500	363	2 (UK[e])	C
12	3,770	2 (77)	Sand/gravel	432,164	1,782	183,752	M	6	384	316	1 (S)	F, C
13	3,735	2 (130–568)	Sand/gravel, sandstone	343,389	1,426	121,769	M	5	431	216	1 (S)	F
14	1,363	2 (100–300)	Sandstone, limestone or dolomite	194,011	479	53,757	M	5	153	99	2 (S)	F

[a]Information obtained from the Wisconsin Department of Natural Resources.
[b]Information obtained from the Public Service Commission of Wisconsin for the year 2006.
[c]The average yield per day was calculated by dividing the annual water volume produced by 365.
[d]M: metal, P: plastic, AC: asbestos-cement.
[e]Unknown.
[f]S: steel; C: concrete.
[g]F: fluoridation; C: corrosion control.
[*]1 ft = 0.3048 meters.

70 μM of deoxynucleotide triphosphate (ProMega), 30 U of RNasin (ProMega), and 100 U of SuperScript II reverse transcriptase (Invitrogen Life Technologies, Rockville, MD, USA). The reaction was incubated at 25 °C for 15 min, 42 °C for 60 min, and 99 °C for 5 min, and then 4 °C until PCR amplification. qPCR was performed directly to quantify adenoviruses.

Quantitative PCR was performed on a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) using PCR mixes prepared with the LightCycler 480 Probes Master Kit (Roche Diagnostics) and fluorescence generated by TaqMan probes (TIB MOLBIOL, Berlin, Germany). 6 μL of DNA or cDNA solution was added to 14 μL of PCR master mix. The sources of the PCR primers and hybridization probes and their final concentrations used in the present study are as follows: enteroviruses: 300 nM forward primer and 900 nM reverse primer (De Leon *et al.* 1990), 100 nM probe (Monpoeho *et al.* 2000); adenoviruses (Cromeans *et al.* 2005) and rotavirus (Gentsch *et al.* 1992): 500 nM primers, 100 nM probe; noroviruses GI (Jothikumar *et al.* 2005) and GII (Ando *et al.* 1995): 250 nM primers, 100 nM probe; and hepatitis A virus (Schwab *et al.* 1995): 700 nM primers, 100 nM probe. Reactions were not multiplexed. All reactions contained 4 mM $MgCl_2$. Amplification reactions started with a hot start polymerase activation step for 10 min at 94 °C, followed by 45 cycles of 15 s at 94 °C and 1 min at 60 °C. Standard curves, reference controls, inhibition controls, negative controls and quality assurance procedures were performed as described previously (Borchardt *et al.* 2004; Lambertini *et al.* 2008). Standard curves were created approximately every 4 months, just before analyzing water samples collected during the previous period. Among the six virus groups tested, qPCR efficiencies ranged between 1.858 and 2.266 ($n = 30$ standard curves). Reference controls, performed with each LightCycler run, were required to fall within ± 0.5 cycles of the original crossing point obtained during standard curve generation in order for the data to be acceptable.

Statistical analysis

One community (ID #8) had recurring coliform contamination problems in its distribution system and chlorinated for the entire study duration. This community was excluded from analysis because the residual disinfectant obviated the effect of the UV intervention to isolate distribution system intrusions and virus concentrations at the taps would have been underestimated. In the other 13 communities no samples were collected during chlorination events. In one community a single tap water sample, out of 42 samples collected in that community, was excluded from analysis as its virus concentration (854 genomic copies/L) was more than an order of magnitude higher than the next highest concentration of all 902 samples and such a high value would have dominated the statistical analysis.

The analysis was carried out at two levels of time aggregation: (1) by 3-month water sampling period, in which the four periods were defined as the time between the day of the first sample collected to the day of the last sample collected in spring 2006, autumn 2006, spring 2007, and autumn 2007, respectively; and (2) by year, where the two years were defined as the time from the first sample collected in spring to the last sample collected in autumn of the same year, 2006 or 2007. The reason for aggregating data by year was two-fold: (1) increase for analysis the number of distribution systems experiencing events; and (2) account for events that occurred in the summer that, nonetheless, if viruses were introduced and entrained in the system's biofilm, could have affected virus levels in the subsequent autumn sampling period.

Two dependent variables were tested for their association with the occurrence of distribution system events: (1) the average all-viruses concentration, calculated by summing the concentrations of all six virus types in genomic copies/L and dividing by the number of samples in a period ($n = 17–24$) or year ($n = 35–48$) taken from a community's distribution system. For those samples positive for more than one virus, the within-sample sum of virus numbers was divided by the sample volume, and this concentration was inputted into the average calculation. Samples with no detected viruses were assigned a zero value and included in the average. (2) The proportion of tap water samples resulting in a positive qPCR signal for any virus type in a community and over the considered time frame. Gamma regression was used to evaluate the association between average virus concentration and distribution system events, whereas binomial regression was used for the proportion of virus-positive samples. When performing gamma regression, average virus concentration values of zero were assigned a value of 10^{-6} genomic copies/L to avoid the undefined function log[zero].

Distribution system events were treated as dichotomous (no event vs. event occurring once or more) with the exception of 'hydrant flushing', which was also treated as a continuous variable and expressed in units of number of days when flushing occurred. Events were counted as distinct when separated by at least one non-event day. Random effects for community and sampling period were included in the models when analyzing data aggregated over 3-month sampling periods. A random effect for year was inserted for analyses where the data were aggregated at the level of the year within each community. All models included fixed effects for a single type of distribution system event and chlorination status. Chlorination status was incorporated in the models to adjust for the potential confounding effect of chlorination on the association between viruses and distribution system events, and was characterized as a dichotomous variable indicating whether distribution system events overlapped with periods of chlorination. Five distribution system events took place while a distribution system was temporarily undergoing chlorination. Whenever an event overlapped with chlorination, it was the only event of that type occurring in the considered time period; hence the summary data point for the period could be unequivocally labeled as 'overlapping with chlorination' or not. A separate unadjusted analysis was also carried out considering chlorination as the independent variable (no chlorination event vs. chlorination occurring once or more). When computing adjusted estimates of a dependent variable for a given value of a distribution system event variable, the weights for the chlorination status regression coefficients were set to the marginal proportions in the source data. All analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Most of the time in the communities, mean virus concentrations were highest in the wells, reduced one to six logs by UV disinfection, and then increased in the distribution system (Figure 1). This concentration increase downstream from UV disinfection demonstrated that viruses were

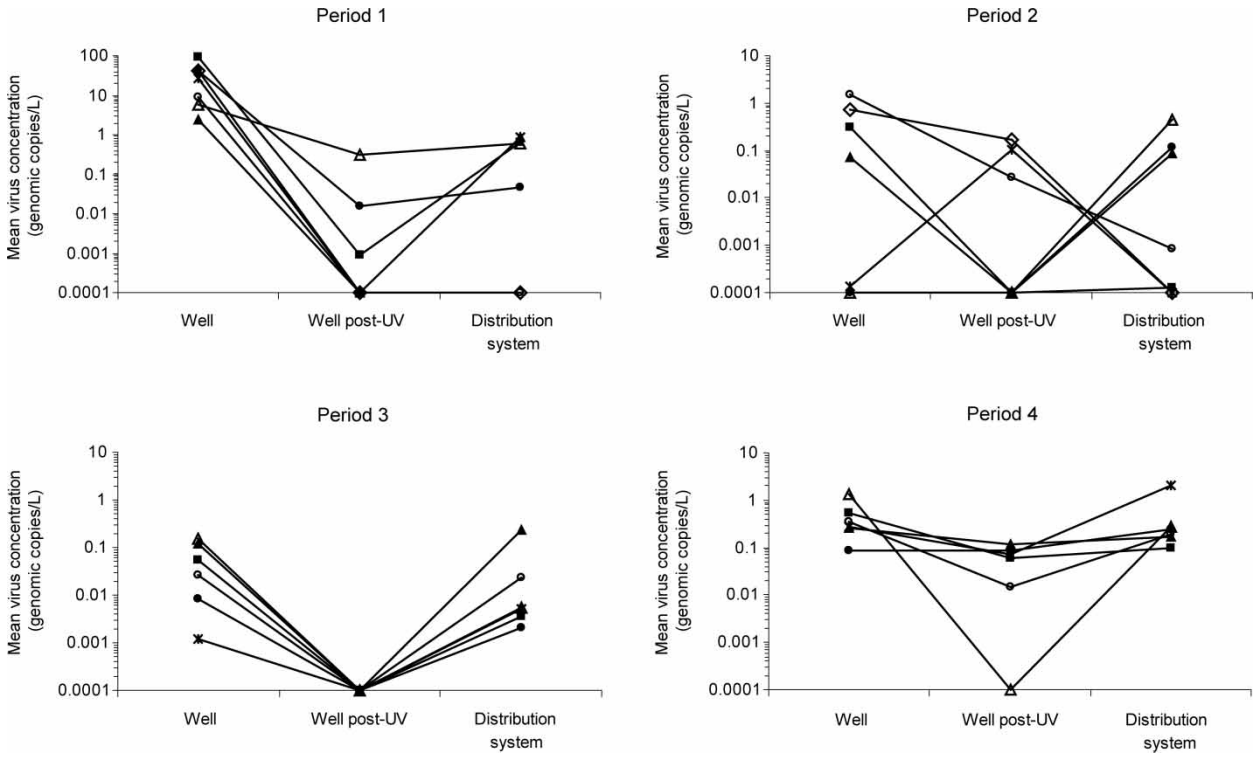

Figure 1 | Mean virus concentrations along the drinking water delivery pathway from municipal wells, immediately following UV disinfection at the wellhead, to in the distribution system as measured at household taps. Each line represents one of the 13 study communities, seven in Year 1 (Periods 1 and 2) and six in Year 2 (Periods 3 and 4). Each symbol represents the same community within a year. Note the change in *y*-axis scale for Period 1. Values of zero genomic copies/L are plotted as 10^{-4} for visualization purposes.

directly entering the distribution system. Real-time qPCR is a sensitive measure of virus inactivation by UV disinfection as the irradiation causes pyrimidine dimers in the virus genome, disrupting the polymerase enzyme from creating target amplicons (Simonet & Gantzer 2006; Eischeid *et al.* 2009; Süß *et al.* 2009). Unlike some bacteria, viruses lack the repair mechanisms for UV-damaged nucleic acids; only by entering a host cell and accessing its enzymes could repair possibly happen (Eischeid *et al.* 2009). De novo creation of qPCR-measurable virus genomes between UV disinfection and household taps was not possible, and any measured increase in virus concentration must have resulted from distribution system intrusions.

Virus intrusions were observed in 12 of the 13 distribution systems. During some periods in some communities the mean virus concentration in the distribution system was the same or lower than the concentration measured immediately following UV disinfection, indicating virus intrusions did not occur. In one community in Period 2 there was never a virus detection in the distribution system, but in one well a single sample was positive for adenovirus, and the companion sample from the post-UV sampling location was also adenovirus-positive, but at a higher concentration, making it appear UV disinfection increased virus concentration (Figure 1).

Considering all communities and periods combined, the mean virus concentration was highest in the wells, reduced two-logs by UV disinfection, and increased one-log in the distribution systems (Table 2). A similar pattern was observed for the detection frequency of all-viruses and each virus type; detection was highest in the wells, lowest post-UV disinfection, and increased again in the distribution systems but not to as high a frequency as in the wells (Table 2). Adenovirus was the most frequently detected virus followed by enterovirus and norovirus genogroup I. Of the 18 post-UV samples that were virus-positive, 17 contained adenoviruses, the virus most resistant to UV disinfection (Yates *et al.* 2006), and the majority of these occurred in Period 4, the time when the change in virus concentration between post-UV and distribution system in several communities was small (Figure 1).

Of the 12 types of distribution system events queried in the questionnaire 10 occurred during the study, and these are reported as the number of communities experiencing events (Table 3(a)) and the number of events across all communities (Table 3(b)). All 13 communities experienced at least one type of event (11 communities experienced events other than hydrant flushing). Two event types queried in the questionnaire never took place during the study and are not reported in Table 3; no routine monitoring samples were found positive for *E. coli*, and no cross-connections were discovered. Five communities had short-term chlorination on eight occasions for emergency reasons (main breaks and routine samples positive for total coliforms) or as a preventive measure during routine maintenance (valve exercising and water tower maintenance).

Of the different event types investigated, adding pipes to the distribution system was the most highly associated with increased virus concentrations, and this association was observed both for data aggregated by 3-month sampling period and by year (Tables 4 and 5). Replacing pipes was not significantly associated with increased virus levels, likely because such events were infrequent. However, when the two similar event types were combined into a single variable, 'pipe replaced or added', this event, too, was significantly associated with higher virus concentrations. The proportion of virus-positive samples did increase when pipes were added or replaced, but the associations were not significant (Tables 4 and 5).

Table 2 | Viruses in wells, in well water immediately following UV disinfection, and in distribution systems (i.e., household tap water) downstream from UV disinfection

Location	No. samples	All viruses % detections	All-viruses concentration (genomic copies/L)				% detections by virus type[a,b]			
			Mean	Median	75% Quartile	Maximum	AdV	EV	NvGI	HepA
Well	182	39.5	6.45	0	0.33	264.4	23.1	13.7	10.4	0
Well post-UV	181	9.9	0.03	0	0	1.77	9.4	1.1	0	0.6
Distribution system	539	20.0	0.27	0	0	17.3	13.4	6.3	1.9	0.6

[a]AdV = adenovirus; EV = enterovirus; NvGI = norovirus genogroup I; HepA = hepatitis A virus.
[b]Only one sample was positive for rotavirus and all samples were negative for norovirus genogroup II.

Table 3(a) | Number of distribution systems (i.e. communities) with an event[a]

Event	Sampling periods 1	2	3	4	Total[b]	Sampling years Year 1	Year 2	Total[c]
Chlorination	4	1	0	0	5	4	1	5
Well shut-down	2	0	1	3	6	2	4	6
Main breaks	2	2	0	0	4	3	2	5
Pipes replaced	0	1	0	1	2	1	2	3
Pipes added	4	1	0	1	6	4	3	7
Pipes replaced or added	4	1	0	2	7	4	4	8
Main cut open	1	1	0	2	4	2	2	4
Water tower maintenance	3	1	1	1	6	3	2	5
Valve exercising	2	3	0	0	5	4	2	6
Total Coliform positives	3	1	1	2	7	3	2	5
Hydrants flushing	5	3	4	4	16	7	6	13

[a]These data were used in the regression analyses (i.e. an event occurred, yes or no, in the distribution system during the considered time period).
[b]The total by sampling period can be greater than the total by sampling year because an event only need occur once to be scored and there are two sample periods within one sample year.
[c]The total number of distribution systems was 13: 7 study communities were considered in year 1 (periods 1 and 2), and 6 communities in year 2 (periods 3 and 4).

The two other event types describing a physical breach in the distribution system, main breaks and cutting open a water main, were not significantly associated with virus levels, although when analyzed by sampling period, cutting open a water main resulted in a four-fold increase in virus concentrations (Table 4).

Whether hydrant flushing was performed or not (dichotomous variable) was not associated with virus levels. However, the extent of hydrant flushing, expressed in units of days when flushing was performed, was highly significantly associated with reduced virus concentrations when data were analyzed over both sampling periods and study years (Tables 4 and 5). Similarly, the proportion of virus-positive samples was weakly negatively associated with days of hydrant flushing when the data were aggregated by year.

Other events were only weakly associated with virus contamination. Water tower maintenance was weakly associated with both the proportion of positive samples and the mean virus concentration over the time frame of sampling periods (Table 4). Valve exercising was weakly associated with mean virus concentration only when data was aggregated at the level of year (Table 5). The event of a well shut-down for maintenance or repairs was marginally significantly associated with lower virus concentrations, although only when aggregating data by study year (Table 5).

Chlorination events, treated as a dichotomous independent variable, were not associated with either the proportion of positive samples or the mean virus concentration. Furthermore, coliform bacteria detections from routine monitoring were not associated with either of the virus outcome measures.

Table 3(b) | Number of events or extent of an event in the distribution systems

Event	Sampling periods 1	2	3	4	Total	Sampling years Year 1	Year 2	Total
Chlorination (# events)	5	2	0	0	7	7	1	8
Well shut-down (# events)	4	0	1	3	8	4	4	8
Main breaks (# events)	3	2	0	0	5	5	2	7
Pipes replaced (ft)*	0	650	0	450	1,100	650	2,350	3,000
Pipes added (ft)	13,662	1,700	0	1,200	16,562	15,362	3,640	19,002
Pipes replaced or added (ft)	13,662	2,350	0	1,650	17,662	1,6012	5,990	22,002
Main cut open (# events)	1	2	0	4	7	3	12	15
Water tower maintenance (# events)	3	1	1	1	6	3	2	5
Valve exercising (days)	21	9	0	0	30	34	2	36
Total coliform detects (# samples)	5	1	1	6	13	6	31	37
Hydrants flushing (days)	38	11	20	26	95	61	63	124

*1 ft = 0.3048 meters.

Table 4 | Association between distribution system events and virus outcome measures. Analysis by sampling period

Event	Outcome: proportion of positive samples		Outcome: mean virus concentration	
	p-value	Outcome ratio (event occurred/no event)[a]	*p*-value	Outcome ratio (event occurred/no event)[a]
Chlorination	0.35	1.42 (0.216/0.152)	0.61	1.59 (0.269/0.168)
Well shut-down	0.62	0.91 (0.151/0.166)	0.26	0.42 (0.086/0.208)
Main breaks	0.94	0.97 (0.158/0.163)	0.54	1.78 (0.289/0.162)
Pipe replaced	0.31	1.28 (0.203/0.159)	0.26	4.21 (0.625/0.148)
Pipes added	0.32	1.25 (0.193/0.154)	0.01	7.04 (0.615/0.087)
Pipe replaced or added	0.21	1.27 (0.193/0.152)	0.01	6.86 (0.577/0.084)
Main cut open	0.45	1.17 (0.185/0.158)	0.18	4.10 (0.560/0.136)
Water tower maintenance	0.11	1.37 (0.205/0.149)	0.18	3.20 (0.416/0.13)
Valve exercising	0.50	0.73 (0.125/0.170)	0.85	0.85 (0.163/0.192)
Total coliform positives	0.61	0.90 (0.150/0.167)	0.20	0.32 (0.073/0.230)
Hydrant flushing	0.75	1.06 (0.166/0.157)	0.82	0.86 (0.174/0.203)
Hydrant flushing (days)[b]	0.30	0.98[c]	0.008	0.78[c]

[a]Ratio of the dependent variable (proportion of positive samples or mean virus concentration) between communities where the considered distribution system event occurred ('event occurred'), and those where the event did not occur ('no event'). For both models, this ratio corresponds to $\exp(\beta)$, the exponent of the estimated parameter β for the distribution system event. Values in parenthesis are the numerator and the denominator of this ratio. All estimates are adjusted for chlorination status (except those in the first row of the table where chlorination status is evaluated). The adjusted numerator and denominator estimates are least squares means with coefficient weights for chlorination status equal to the marginal proportions in the source data.

[b]Results in this row refer to the 'Hydrant flushing' variable treated as an integer, expressed in units of days.

[c]Ratio of estimated outcome values for a 1-day difference in hydrant flushing.

Table 5 | Association between distribution system events and virus outcome measures. Analysis by year

Event	Outcome: proportion of positive samples		Outcome: mean virus concentration	
	p-value	Outcome ratio (event occurred/no event)[a]	*p*-value	Outcome ratio (event occurred/no event)[a]
Chlorination	0.64	0.88 (0.174/0.198)	0.99	0.99 (0.276/0.278)
Well shut-down	0.65	0.90 (0.178/0.198)	0.04	0.30 (0.125/0.410)
Main breaks	0.83	1.05 (0.195/0.185)	0.56	1.44 (0.341/0.237)
Pipe replaced (ft)[*]	0.16	1.37 (0.237/0.172)	0.95	0.95 (0.267/0.281)
Pipes added (ft)	0.38	1.23 (0.207/0.168)	0.05	3.28 (0.410/0.125)
Pipe replaced or added (ft)	0.22	1.39 (0.211/0.152)	0.11	2.96 (0.374/0.126)
Main cut open	0.16	1.37 (0.230/0.168)	0.73	1.30 (0.331/0.254)
Water tower maintenance	0.28	1.27 (0.217/0.171)	0.64	1.43 (0.340/0.239)
Valve exercising	0.75	1.08 (0.197/0.182)	0.17	2.19 (0.397/0.181)
Total coliform positives	0.91	1.04 (0.193/0.186)	0.61	0.63 (0.205/0.328)
Hydrant flushing (days)[b,d]	0.10	0.97[c]	0.001	0.85[c]

[a,b] and [c] See footnote in Table 4.

[d]Dichotomous (yes/no) 'Hydrant flushing' variable was not analyzed because all communities flushed their hydrants at least once during the year (Table 3(a)), making comparisons between flushing and non-flushing communities impossible.

[*]1 ft = 0.3048 meters.

DISCUSSION

The occurrence of transient negative pressures in distribution systems (Kirmeyer *et al.* 2001), the presence of pathogens in the trench material surrounding underground distribution system pipes (Karim *et al.* 2003), the contribution of distribution system events to coliform-positive samples (Besner *et al.* 2007), and the association of

distribution system events with gastrointestinal illness (Nygård *et al*. 2007) all strongly support the concept of distribution system vulnerability to direct contamination. Our study now provides clear evidence that human pathogenic viruses can directly enter into distribution systems, and that the level of virus contamination was related to common distribution system events.

Pipe installation in the distribution systems was significantly associated with virus contamination. These results are consistent with Besner *et al*. (2007) who, in a study involving several municipalities, found that coliform and heterotrophic bacteria counts were associated with pipe addition, cleaning, and repair. Soil or water carrying pathogenic organisms can inadvertently enter the exposed pipes, and if not inactivated, the contaminants can then spread to other sections of the system. Fecal contamination has been observed in soil and water samples adjacent to drinking water lines (Karim *et al*. 2003; Besner *et al*. 2008). Pathogenic organisms can also be present in new pipe before installation (Haas *et al*. 1999), and can contaminate the system if the new pipe is not effectively disinfected. Such problems are known to water utility managers, who report unsanitary practices during water system construction as a leading cause of contamination (Haas 1998). In addition to direct entry, rapidly closing off a section of the system to carry out any repair or maintenance work can cause a sudden shift in water velocity, resulting in a pressure wave and possibly a low or negative pressure transient (Karim *et al*. 2003; LeChevallier *et al*. 2003; Gullick *et al*. 2004). During such an event the pressure gradient can cause water to flow from the soil surrounding the pipe into the system through leaks, non-sealing joints, air-valve vaults, meter boxes, or other openings. A detailed investigation of distribution network integrity was outside the scope of the present study, but it is recognized that these intrusion routes are commonly present in distribution systems (Kirmeyer *et al*. 2001). Adding pipes appeared to be a greater threat to water quality than replacing existing pipes. As standard practices to prevent contamination during the addition of new pipes are the same as for the replacement of existing pipes, this finding may result from the disproportionate length of new pipes added to the distribution systems during the study, more than an order of magnitude higher than the length of existing pipes replaced.

Reduced virus levels were highly significantly associated with the number of days a community performed hydrant flushing. All 13 communities flushed hydrants, but only two communities practiced chlorination during flushing events, and as chlorination was accounted for in the regression analysis, it appears the physical flushing process was responsible for reducing virus concentrations. Flushing to remove soft deposits and particulate matter from the system can improve microbial water quality in two ways: (1) by eliminating a 'habitat' where microbes can accumulate or grow; and (2) by removing organic matter and ferrous iron particles, which can reduce the effectiveness of chlorine-based disinfectants (Lehtola *et al*. 2004). Unidirectional flushing of the distribution system has been observed to improve water quality, assessed in terms of heterotrophic bacteria levels, for up to 6 months to 2 years (Lehtola *et al*. 2004; Barbeau *et al*. 2005). Deposits removed by flushing were observed to contain heterotrophic bacteria, as well as other organisms (Gauthier *et al*. 1999), although no evidence is available on virus occurrence on such deposits, or the effectiveness of flushing to remove viruses. Our results confirm previous findings that distribution system flushing improves microbial water quality, expanding the available evidence to include human enteric viruses.

Water tower maintenance was weakly associated with increased viral contamination when the data were aggregated over 3-month study periods. Water towers are a recognized pathway for microbial contamination of distribution systems, particularly from bird droppings (Missouri Department of Natural Resources 2006). Water tower maintenance and cleaning should improve water quality in the distribution system downstream (Marterl *et al*. 2005). However, these operations by themselves can introduce contaminants, either directly or indirectly through rapid changes in water pressure. In the present study, all episodes of water tower cleaning were performed as regular maintenance (painting the interior, draining, and opening for access), not in reaction to water quality problems. During one event of water tower maintenance out of a total of six events, the system was chlorinated, possibly masking virus contamination. However, chlorination was accounted for in the regression model. It is possible contaminants introduced or mobilized by maintenance work were to some extent offset by the water quality benefits of removing

particulate matter, biofilms, and deposits from the tower, similar to the benefits obtained by flushing mains.

Valve exercising was not associated or only weakly associated with virus contamination. This practice involves opening or closing valves along the distribution network to ensure they are functional, which could alter flow and pressure patterns across the system, mobilizing accumulated microorganisms or giving rise to low pressure transients (Vitanage *et al.* 2004). In the present study, only four communities exercised valves, possibly limiting statistical power in the regression analysis.

Well shut-downs were significantly, but inversely, associated with virus concentrations when data were aggregated by year. This result is surprising because, as with other events that trigger transient negative pressures, it is expected water quality would be impaired not improved. Well pumps in the study communities were turned on and off daily as part of routine operations and any single shut-down event for maintenance, the event of interest here, would not be expected to behave any differently than the daily on-off cycling of the pumps. Wells were not shut down for contamination issues, so improved water quality cannot be explained by removing contamination sources. Chlorination did not seem to confound the relationship; there was only one out of eight well shut-down events where chlorination, applied between the shut-down and water sample collection, could have caused the samples to be misleadingly virus-negative. It is possible that well shut-down events were, by chance, positively or negatively correlated with other distribution system events, perhaps unmeasured, that were mechanistically linked to virus levels.

Total coliform detections by routine monitoring were not associated with either virus outcome measure, consistent with previous reports that indicator bacteria are not good surrogates for the presence of human enteric viruses (Ashbolt *et al.* 2001). However, in a study relating total coliform detection to operation and management activities in distribution systems, between 9 and 45% of coliform-positive samples could be explained by distribution system events (Besner *et al.* 2007), suggesting this indicator has some value in identifying microbial intrusions. In the present study, four of the five communities with coliform detections promptly followed-up with system-wide chlorination. If viruses and coliforms had entered the distribution systems at the same time, virus inactivation by chlorination may have accounted for the absence of an association. On the other hand, the virus outcome measures reflect virus entry over time (3 or 6 months of virus samples per distribution system, aggregated by 3-month study periods or by study years respectively), and if coliform detections are a general proxy for system vulnerability, a significant association would be expected.

Main breaks were not associated with virus contamination though they would appear to pose risks similar to adding or replacing pipes or even greater risks because of the unpredictable nature of the event. Our results appear to be inconsistent with the findings of Nygård *et al.* (2007), who observed a positive correlation between main breaks and the rate of gastrointestinal illness. In our study, an association may not have been observed because: (1) There were too few distribution systems with main breaks; and (2) Main break severity was not quantified (e.g., how much time elapsed between a break, its detection, and its repair), which could have significant repercussions on the extent of water contamination (Geldreich *et al.* 1992).

The drinking water systems considered in this study, serving populations between 1,000 and 8,000 inhabitants and using groundwater sources, are common in the US. Systems serving less than 10,000 inhabitants constitute 92% of all community water systems, while 78% of community water systems rely on groundwater (US Environmental Protection Agency 2009). The study utilities did not apply any disinfection treatment prior to water distribution, which is not an uncommon practice in the USA as 10% of the US population served by community groundwater systems receives non-disinfected water, while 57% receives water that is either non-disinfected or treated with a method that achieves less than a 4-Log reduction in viruses (US Environmental Protection Agency 2006). Also, the study communities are typical in their microbial contamination levels, presenting a frequency of total coliform detections by routine monitoring that is consistent with national averages observed in US communities of a similar size (Michael Messner, U.S. EPA, personal communication). The age and materials of the distribution systems in the study communities are common in US systems (National Research Council 2006). The occurrence of distribution system events varied from community to community, with very few communities (2 out of 13) reporting no event

besides hydrant flushing and valve exercising. However, the reported rate of main breaks in the study was 0.01 breaks per mile per year, considerably lower than the estimated US average of 0.27 breaks per mile per year (Kirmeyer *et al.* 1994).

Several limitations must be considered when interpreting the results of the present study. While UV irradiation will decrease the number of viral genomes that are amplifiable by qPCR (Simonet & Gantzer 2006; Eischeid *et al.* 2009; Süß *et al.* 2009), insofar as UV disinfection of the well water was not 100% effective in blocking amplification of the target nucleic acid sequence, the number of viral genomic copies attributed to distribution system intrusions might have been overestimated. This happened most likely in Period 4 when adenoviruses, the most UV resistant human enteric virus (Yates *et al.* 2006), were detected in the post-UV samples. Another limitation may have been the water sampling scheme, which was designed to characterize average system-wide viral contamination in 14 distribution systems. If it had been feasible, sampling shortly after and near the site of an event may have allowed more robust analysis of associations between distribution system events and virus concentrations. For example, water quality may be affected only locally for a short time after a main break, perhaps explaining why an association between main breaks and virus contamination was not observed. The distribution system questionnaire presented another limitation. While utility managers maintained written records of system events, in some cases to comply with State regulations, recall error may still have been possible. Lastly, it was not possible to measure all factors that may be important in distribution system contamination, for example pipe age, water residence time, or number of leaks.

CONCLUSIONS

While many events associated with the maintenance and repair of drinking water distribution systems may contribute to virus contamination, not all events pose the same risk. The addition of pipe was found to be significantly associated with an increase in virus concentration in drinking water distribution systems. Conversely, the frequency of hydrant flushing was found to be significantly associated with the reduction of virus concentration in drinking water distribution systems. As small systems relying on groundwater are the most common type of community water systems in the US, these findings are pertinent to a large portion of the national water infrastructure. Moreover, since US water systems are aging faster than they are upgraded and are approaching the end of their design life, the illness burden associated with the maintenance and repair of distribution systems may increase in the coming decades.

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