

## Global occurrence of Torque teno virus in water systems

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### ABSTRACT

Bacterial indicator organisms are used globally to assess the microbiological safety of waters. However, waterborne viral outbreaks have occurred in drinking water systems despite negative bacterial results. Using viral markers may therefore provide more accurate health risk assessment data. In this study, fecal, wastewater, stormwater, surface water (fresh and salt), groundwater, and drinking water samples were analyzed for the presence or concentration of traditional indicators, innovative indicators and viral markers. Samples were obtained in the United States, Italy, and Australia and results compared to those reported for studies conducted in Asia and South America as well. Indicators included total coliforms, *Escherichia coli*, enterococci, male-specific coliphages, somatic coliphages and microviridae. Viral markers included adenovirus, polyomavirus, and a potential new surrogate, Torque teno virus (TTV). TTV was more frequently found in wastewaters (38–100%) and waters influenced by waste discharges (25%) than in surface waters used as drinking water sources (5%). TTV was also specific to human rather than animal feces. While TTV numbers were strongly correlated to other viral markers in wastewaters, suggesting its utility as a fecal contamination marker, data limitations and TTV presence in treated drinking waters demonstrates that additional research is needed on this potential viral indicator.

**Key words** | fecal indicators, Torque teno virus, viral pathogen monitoring

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### INTRODUCTION

Indicator organisms are used to establish potential risk from fecal contamination in drinking waters. The United States Environmental Protection Agency, the Council of European Communities, and the World Health Organization all specify bacterial indicators (e.g., fecal coliforms, *Escherichia coli*) in regulations and guidelines. However, waterborne viral outbreaks have occurred in treated drinking water systems where the systems were in compliance with regulations (Craun *et al.* 2006). In fact, many epidemiological studies fail to show a relationship between viral pathogens and bacterial

indicators in environmental systems and through treatment processes (Ashbolt *et al.* 2001; Hamza *et al.* 2011; McQuaig & Noble 2011). Viral and protozoan pathogens are known to be more persistent in the environment than indicator bacteria (Wu *et al.* 2011; Sidhu *et al.* 2012a, b). In treatment systems, correlations between bacteria and viruses are often lacking as a result of differences in physical removal and inactivation kinetics (Payment *et al.* 1985; Blatchley *et al.* 2007; Carducci *et al.* 2008; Shin & Lee 2010; Lee & Sobsey 2011).

Using a virus to predict viral pathogen risk may overcome these limitations (Kopecka *et al.* 1993; Jiang *et al.* 2001; Abbaszadegan *et al.* 2008). Coliphages resemble many enteric viruses in their physical structure and morphology, and can be detected by plaque assay (Ashbolt *et al.* 2001). Some groups are found in high concentrations in wastewaters, are relatively resistant to chlorination, and can be used to distinguish between fecal pollution of human and animal origin (Leclerc *et al.* 2000; Scott *et al.* 2002; Long *et al.* 2005). However, other groups are rarely found in individual human feces and can replicate in the environment (Muniesa & Jofre 2004; Payment & Locas 2011). An alternative to indicators is direct pathogen monitoring which provides information on actual risk from a particular virus but may not be indicative of risks from viruses in general. Noroviruses are the most common cause of acute non-bacterial gastroenteritis and based on structural similarities, have been presumed to have similar persistence in the environment and through treatment as other viruses (Bae & Schwab 2008; Park & Sobsey 2011). However, noroviruses are found in high concentrations in cold months but typically not in warm months, and correlations between noroviruses and fecal indicator bacteria are often lacking (Haramoto *et al.* 2006; Westrell *et al.* 2006). Human adenoviruses, which cause respiratory or gastrointestinal illness, have been detected in surface and groundwaters, wastewaters and finished drinking waters, though correlations between this pathogen and indicator bacteria or coliphages were often not present (Jiang & Chu 2004; Carducci *et al.* 2008; Ogorzaly *et al.* 2010; Okoh *et al.* 2010). In addition, a lack of correlation with other pathogenic viruses indicates that data on adenoviruses may only provide risk from this one pathogen rather than viral pathogens in general. A potential viral surrogate that has not been fully examined is Torque teno virus (TTV).

TTV is a small, non-enveloped, single stranded DNA virus that was first isolated in Japan from the serum of a patient with post-transfusion hepatitis (Nishizawa *et al.* 1997). It was later detected in blood samples from patients in several other countries, including the United States, France, Italy, and Brazil (Leary *et al.* 1999; Biagini *et al.* 2000; Bendinelli *et al.* 2001; Maggi *et al.* 2001; Bassit *et al.* 2002; Diniz-Mendes *et al.* 2004; Devalle & Niel 2005). TTV appears to be present ubiquitously in humans and elicits

seemingly innocuous infections. TTV in humans can be found throughout the body including in blood and feces, and replicates actively in most tissues and organs (Maggi & Bendinelli 2009; Okamoto 2009a). TTV infections have been identified throughout the world, with highest infection rates in countries with poor sanitation (Maggi *et al.* 2011). Research suggests that TTV may possibly cause chronic life-long viremias in most people regardless of age, health status, and other variants.

TTV is excreted in the feces and has been detected in wastewater streams in multiple countries. In Japan, TTV DNA was detected in 12 of 12 wastewater influent samples, with a geometric mean concentration of  $1.7 \times 10^4$  genomic copies/liter (Haramoto *et al.* 2008). The concentration of TTV DNA in the influent samples showed no clear seasonal pattern, suggesting that TTV infections occur year-round. Vaidya *et al.* (2002) found raw sewage prevalence of TTV DNA was statistically similar to the prevalence of hepatitis E virus RNA and hepatitis A virus RNA. Diniz-Mendes *et al.* (2008) found a TTV positivity rate of 92.3% in polluted streams of Brazil. In contrast, Hamza *et al.* (2011) found that TTV was not a suitable indicator of fecal contamination in river water in Germany resulting from low detection rates. In Brazil, Vecchia (2009) found that TTV was sporadic in surface water samples. For a new water quality indicator to be widely applied as coliforms or enterococci are currently, their relevance to geographical region and water type must be understood. Therefore, the purpose of this study was to evaluate TTV as an indicator of fecal contamination in water systems from three continents. TTV presence and/or concentration were compared to bacterial fecal indicators, coliphages and enteric viruses. Data were collected in the United States, Italy and Australia and compared to those published for Asia and South America to conduct a preliminary assessment of the suitability of this potential viral surrogate.

## METHODS

### Sample collection

TTV occurrence was evaluated by collecting and analyzing animal feces, wastewaters, stormwaters, surface waters (fresh and salt), groundwaters, and finished drinking

waters. Samples were collected and analyzed by Worcester Polytechnic Institute (Worcester, MA, USA), the University of Wisconsin-Madison (Madison, WI, USA), the University of Pisa (Pisa, Italy), and the Commonwealth Scientific and Industrial Research Organization (CSIRO) (Brisbane, Australia). Appropriate positive and negative controls were included for all tests. Samples were diluted or concentrated as appropriate to achieve acceptable detection limits. The samples analyzed are summarized in Table 1.

In the United States, fecal, wastewater and drinking water samples were collected from four different regions (Northeast, South, Midwest, and West) in multiple seasons. Fresh fecal samples ( $n = 75$ ) included five animal groups: chicken, dog, equine (horse and donkey), rabbit, and ruminant (cow, sheep, goat, and llama). Animals were monitored by the sampler and feces were collected in sterile containers immediately after defecation. Wastewater, surface water, ground water and finished drinking water samples were collected from municipalities. Wastewater samples (1 L) included influent and effluent samples ( $n = 25$ ). Water samples (20 L) ( $n = 39$ ) included raw surface waters, raw groundwaters and treated drinking waters

collected from public water supply distribution systems (Plummer *et al.* 2014).

In Italy, wastewater and surface water samples were collected from the greater Pisa area, localized in the Tuscany region. Samples ( $n = 24$ , first sampling period;  $n = 58$ , second sampling period) were collected from the city of Pisa activated sludge wastewater treatment plant (1 L influent and 10 L effluent). Surface water samples (10 L) were collected from the river Fiume Morto ( $n = 12$ ) downstream from the city of Pisa wastewater treatment plant discharge and from a seawater outfall ( $n = 12$ ) (Carducci *et al.* 2006; Verani *et al.* 2006).

In Australia, wastewater and stormwater samples were collected from the greater Brisbane area. Influent (1 L) and effluent (20 L) wastewaters were collected from the Luggage Point, Oxley Creek, and Bundamba wastewater treatment facilities ( $n = 44$ ). Stormwater samples ( $n = 40$ ) were collected from two sites (Fitzgibbon and Markerston catchment areas) in Brisbane, Australia during three storm events. Samples were collected using automated sampling infrastructure (ISCO 6700 or equivalent) triggered by automated flow measurement (Doppler flowmeter or weir) (Toze *et al.* 2012; Sidhu *et al.* 2013).

**Table 1** | Summary of sampling events

Source	Location	Dates (MM/YY)	Total samples	Individual samples	Sample type
Fecal	USA	06/10 to 04/11	75	10	Chicken
				15	Dog
				22	Equine
				3	Rabbit
				25	Ruminant
Wastewater	USA	06/10 to 04/11	25	13	Influent
				12	Effluent
	Italy	04/04 to 03/05	24	12	Influent
				12	Effluent
				29	Influent
	Australia	03/07 to 04/08	58	29	Effluent
				22	Influent
Stormwater	Australia	01/10 to 06/10	44	22	Influent
				22	Effluent
Surface water	Australia	01/12 to 03/12	40	16	Markerston catchment
				24	Fitzgibbon catchment
Surface water	USA	05/11 to 03/12	15	15	Fresh surface water
	Italy	05/04 to 04/05	12	12	River water
	Italy	05/04 to 04/05	12	12	Seawater
Groundwater	USA	05/11 to 03/12	4	4	Raw groundwater
Drinking water	USA	05/11 to 03/12	20	20	Distribution system

## Bacteria enumerations

Data were collected for three bacterial indicators: total coliforms, *E. coli*, and enterococci. All enumerations were conducted in accordance with accepted methodologies and with appropriate quality control/quality assurance. In the United States, total coliforms and *E. coli* were enumerated using Standard Methods 9223 (APHA *et al.* 2012) with Coli-*lert*<sup>®</sup> (IDEXX, Westbrook, ME) in the multiple well format (Quanti-Tray<sup>®</sup>, IDEXX, Westbrook, ME, USA) and yielded a most probable number (MPN) of the target organisms per 100 mL. Dilutions and concentrations were performed as needed (Plummer & Long 2013). In Italy, *E. coli* and enterococci concentrations were determined by Bio-Rad miniaturized methods (Bio-Rad Laboratories, Milan, Italy) using MUG/EC microplates and MUD/SF microplates, respectively. These methods provide a MPN of the indicators in accordance with ISO 9308-3 for *E. coli* (ISO 1998a) and ISO 7899-1 for enterococci (ISO 1998b) (Bofill-Mas *et al.* 2010). In Australia, fecal bacteria (*E. coli* and enterococci) were quantified using the membrane filtration technique. Samples (1 and 10 mL) were filtered through 0.45 µm nitrocellulose (Millipore, Billerica, MA, USA) membranes which were placed on Chromocult<sup>™</sup> coliform agar (Merck, München, Germany) for *E. coli* and Chromocult<sup>™</sup> Enterococci agar (Merck, München, Germany) for enterococci. The plates were incubated overnight at 37 °C and then typical colonies were counted providing colony forming unit counts (Sidhu *et al.* 2012a, b).

## Coliphage enumeration

Coliphages were enumerated in accordance with accepted methodologies and with appropriate QA/QC procedures. The US samples were analyzed for somatic and male-specific coliphages by EPA Method 1602, the single layer agar method (US EPA 2001). *E. coli* CN-13 (ATCC 700609, Manassas, VA; resistant to nalidixic acid) and *E. coli* F-amp (ATCC 700891, Manassas, VA; resistant to streptomycin and ampicillin) were used as hosts for somatic and male-specific coliphages, respectively. Samples were supplemented with magnesium chloride, log phase host bacteria, and agar. Plates were incubated overnight at 36 °C and examined for plaque forming units (PFU)/100 mL. In Italy, somatic

coliphages were enumerated by the ISO double agar layer plaque assay method using *E. coli* C (ATCC 13706, Manassas, VA) as the host strain (ISO 1999). The sample, host and top layer agar were mixed and added to a plate with a hard layer of agar. The plates were incubated overnight at 36 °C and counted for PFU/100 mL. In Australia, somatic coliphages (Microviridae family) were enumerated using quantitative polymerase chain reaction (qPCR) with Bio-Rad iQ<sup>™</sup>5 (Bio-Rad Laboratories, Hercules, CA, USA) using iQ<sup>™</sup> Supermix real-time PCR kit. Details are provided in Sidhu *et al.* (2012a, b).

## Viral enumeration

In the United States, drinking water samples were hollow-fiber ultrafiltration (HFUF) concentrated through Asahi REXEED-21S filters with a 30 kDa molecular weight cutoff. HFUF concentrates and wastewaters were concentrated using polyethylene glycol (PEG) precipitation, and 0.25 g of fecal samples were extracted directly. Multiple PCR methods were used to enumerate viruses. Extraction of nucleic acid was accomplished by bead beating (PEG concentrates and solid samples) and the use of a clean-up kit (PowerSoil<sup>®</sup> DNA Isolation Kit, MO BIO Laboratories, Carlsbad, CA, USA) to reduce inhibitor concentrations. For TTV, amplification of target ssDNA was conducted using a traditional PCR assay modified from that reported by Carducci *et al.* (2008). All positive TTV samples and a selected number of negative TTV samples were analyzed for the presence of human adenovirus. A qPCR assay was developed with primer/probe sets, master mix conditions, and thermocycler program modified from those described by Jothikumar *et al.* (2005). Full details of the US methods are provided in Plummer & Long (2013).

In Italy, water samples were concentrated using two-stage tangential flow ultrafiltration. After prefiltration on polypropylene membranes, the samples were filtered through a polysulphone membrane with a 10,000 MW exclusion size. The samples were reconcentrated with a mini-ultrasette apparatus and washed using 15–20 mL of 3% beef extract at pH 9, obtaining a concentrated sample of 40 mL at pH 7. The concentrated samples were decontaminated with chloroform and the nucleic acids were extracted with QIAamp DNA kit (QIAGEN, Hilden, Germany). The extracted nucleic acids were assayed with qualitative and quantitative biomolecular

tests (nested PCR and real-time PCR) according to published protocols to reveal the presence and the titer of adenoviruses and TTV viral genomes (Carducci *et al.* 2009).

In Australia, samples were analyzed by qPCR for TTV, adenovirus, polyomavirus, and microviridae. The samples were concentrated using Hemoflow HF80S dialysis filters (Fresenius Medical Care, Lexington, MA, USA). Samples were pumped with a peristaltic pump in a closed loop with high-performance, platinum-cured L/S 36 silicone tubing. Samples were concentrated to approximately 100 mL and further concentration of sample was carried out by JumboSep with 100 K MWCO filters (Pall, Melbourne, Australia) to a final volume of approximately 10 mL (Sidhu *et al.* 2013). Nucleic acid was extracted from 200  $\mu$ L of each concentrated sample using the QIAGEN DNeasy Blood and Tissue kit (QIAGEN Inc., Valencia, CA, USA) per manufacturer instructions, and stored at 80 °C until processed. Amplifications were performed in 25  $\mu$ L reaction mixtures using iQ Supermix (Bio-Rad Laboratories, Berkeley, CA, USA). The PCR mixture contained 12.5  $\mu$ L of Supermix, 400–500 nM each primer, 400–600 nM corresponding probe, and 3  $\mu$ L of template DNA (Sidhu *et al.* 2013).

### Statistical analyses

Statistical analyses were conducted using IBM's statistical package for the social sciences product, Version 17.0. Analysis of variance (ANOVA) was conducted on the quantitative data sets to evaluate differences based on season and country. Correlation analyses were conducted to determine correlations among the bacterial indicators, coliphages and viruses. The Spearman Rank correlation analysis was used for quantitative data sets, and the point biserial correlation for binary data sets. Statistical analyses were conducted for data sets with over 20 results. All analyses were conducted at the 95% confidence level ( $\alpha = 0.05$ ).

## RESULTS

### Bacterial indicator results

Total coliforms, *E. coli* and/or enterococci were enumerated in all samples (data not shown). Results showed expected

patterns. Fecal samples had a wide range of indicator concentrations, from below detection limits to  $10^8$  MPN per gram. Among water samples, raw wastewaters had the highest indicator concentrations (up to  $10^8$  MPN per 100 mL total coliforms and  $10^6$  MPN per 100 mL *E. coli*). Levels decreased by two to three orders of magnitude through treatment, with bacteria levels in the hundreds of MPN per 100 mL in final wastewater samples prior to disinfection. Next, stormwater samples from Australia had lower fecal bacterial levels than wastewater, with concentrations in the tens to  $10^4$  CFU/100 mL. Seawater and surface water samples were variable depending on overall water quality. Samples downstream of a wastewater treatment plant outfall had *E. coli* in the thousands per 100 mL, compared to drinking water sources in the tens per 100 mL. Finished drinking waters were all negative for *E. coli*, as expected.

### Coliphage results

Coliphage concentrations in fecal samples, wastewaters and waters are summarized in Table 2. Male-specific and somatic coliphages were detected in approximately half of the fecal samples with 41 of 75 samples (54.7%) below detection limits for male-specific coliphages and 32 of 75 (42.7%) below detection limits for somatic coliphages. For fecal samples with detectable levels of coliphages, the maximum male-specific and somatic coliphage concentration were observed in chickens ( $2.0 \times 10^6$  PFU/g and  $2.5 \times 10^7$  PFU/g, respectively).

The US raw wastewater samples had maximum concentrations of  $3.0 \times 10^5$  and  $1.6 \times 10^5$  PFU/100 mL for male-specific and somatic coliphages, with median concentrations on the order of  $10^4$  PFU/100 mL. Coliphage reductions through treatment varied significantly, with approximately 4 log reduction for male-specific coliphages and no significant reduction for somatic coliphages. The raw wastewater samples from Italy had a maximum somatic coliphage concentration of  $1.0 \times 10^7$  PFU/100 mL, with a median of  $2.4 \times 10^6$  PFU/100 mL and a median reduction of two orders of magnitude through treatment. Stormwater samples had a median of 90 PFU/100 mL and a maximum of 870 PFU/100 mL for somatic coliphages.

Coliphage concentrations in surface waters from the USA were much lower than samples from Italy. The

**Table 2** | Coliphage indicator data

Country	Source	Sample type	n	Male-specific coliphage (PFU/g or 100 mL)			Somatic coliphage (PFU/g or 100 mL)		
				Min	Median	Max	Min	Median	Max
USA	Fecal	Chicken	10	BDL	BDL	$2.0 \times 10^6$	BDL	$2.0 \times 10^4$	$2.5 \times 10^7$
USA		Dog	15	BDL	BDL	170	BDL	6.1	$1.8 \times 10^4$
USA		Equine	22	BDL	9.2	$2.9 \times 10^4$	BDL	BDL	$1.0 \times 10^5$
USA		Rabbit	3	BDL	370	$4.9 \times 10^4$	BDL	BDL	$3.0 \times 10^5$
USA		Ruminant	25	BDL	BDL	$5.2 \times 10^4$	BDL	180	$8.4 \times 10^4$
USA	Wastewater	Raw	13	$2.2 \times 10^5$	$9.0 \times 10^4$	$3.0 \times 10^5$	733	$4.0 \times 10^4$	$1.6 \times 10^5$
USA		Final	12	BDL	120	760	170	$1.4 \times 10^3$	$5.1 \times 10^5$
Italy		Raw	41	NT	NT	NT	$4.0 \times 10^5$	$2.4 \times 10^6$	$1.0 \times 10^7$
Italy		Final	41	NT	NT	NT	$1.0 \times 10^3$	$1.9 \times 10^4$	$2.0 \times 10^6$
Australia	Stormwater	Stormwater	40	NT	NT	NT	1.0	91	870
Italy	Surface water	Sea	12	NT	NT	NT	0.10	250	700
Italy		River	12	NT	NT	NT	$4.6 \times 10^4$	$1.7 \times 10^5$	$4.6 \times 10^5$
USA	Groundwater	Fresh	15	BDL	BDL	1.8	BDL	BDL	5.8
USA		Raw	4	BDL	BDL	1.0	BDL	BDL	0.34
USA		Drinking water	Distribution	20	BDL	BDL	190	BDL	BDL

NT – not tested, BDL – below detection limit.

median and maximum concentration of somatic coliphages in the seawater samples ( $n = 12$ ) were 250 and 700 PFU/100 mL. In surface water samples in Italy, median and maximum concentrations were on the order of  $10^5$  PFU/100 mL. For US surface water samples, 80.0% and 46.7% were below detection limits for male-specific and somatic coliphages, and for ground water samples, 75.0% were below detection limits for both male-specific and somatic coliphages. For samples with detectable levels, concentrations were in the tenths to ones of PFU/100 mL. US surface and groundwater samples were all drinking water sources. Similarly, the distribution system samples had a high percentage of non-detects (80.0%) for coliphages.

## Virus results

Quantitative results for viruses are summarized in Table 3. TTV was above detection limits in 36 of 58 (62.0%) wastewater samples in Italy with a maximum concentration of  $3.6 \times 10^5$  genomic copies per mL in raw wastewater and a median reduction of an order of magnitude through treatment. Stormwater and wastewater samples from Australia were quantified for TTV ( $n = 22$ ), adenovirus ( $n = 44$ ), polyomavirus ( $n = 44$ ),

and microviridae ( $n = 22$ ). All samples had quantifiable virus numbers, with maximum TTV in raw wastewater of  $2.4 \times 10^5$  genomic copies per mL which was reduced by approximately two orders of magnitude through treatment. Maximum adenovirus, polyomavirus, and microviridae concentrations were all on the order of  $10^3$  genomic copies per mL in treated effluent with similar removals through treatment. Stormwater samples tested for viral markers were primarily in the one and tens of genomic copies per mL with maximum concentrations of 13, 9.1, and 33 genomic copies per mL for TTV, adenovirus, and polyomavirus, respectively.

In addition to the quantitative virus data, presence/absence testing was conducted using different methodologies for selected samples. TTV was present in three of 76 fecal samples (4.0%). In wastewaters, TTV was present in 38–49% of samples, depending on sample type (raw versus final) and location (country). Surface water detection was rare, with three of 12 river waters in Italy positive for TTV; however, no seawater samples in Italy and no surface water samples in the USA had TTV. There was one (of four) groundwater samples and four (of 20) drinking water samples positive for TTV in the USA. Adenovirus was not found in any fecal samples, surface waters, groundwaters or drinking

**Table 3** | Virus data

Country	Source	Sample type	n	TTV (genomic copy per mL)			Adenovirus (genomic copy per mL)			Polyomavirus (genomic copy per mL)			Microviridae (genomic copy per mL)		
				Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
(a) Quantitative data															
Italy	Wastewater	Raw	29	BDL	697	$3.6 \times 10^5$	NT	NT	NT	NT	NT	NT	NT	NT	NT
Italy		Final	29	BDL	17	$2.4 \times 10^4$	NT	NT	NT	NT	NT	NT	NT	NT	NT
Australia		Raw	11–22	130	250	$2.4 \times 10^5$	110	510	$9.1 \times 10^5$	410	$1.0 \times 10^5$	$2.2 \times 10^5$	$1.2 \times 10^5$	$2.2 \times 10^5$	$5.5 \times 10^5$
Australia		Final	11–22	0.19	0.90	3.9	0.18	0.83	6.0	0.077	0.26	1.2	0.090	0.43	3.8
Australia	Stormwater	Stormwater	24–40	0.010	2.2	13	0.0040	0.22	9.1	0.010	0.010	33	NT	NT	NT
Country	Source	Sample type	n	TTV positive		Adenovirus positive									
				Number	Percentage (%)	Number	Percentage								
(b) Presence/absence data															
USA	Feces	Chicken	10	1	10	0	0%								
USA		Dog	15	2	13	0	0%								
USA		Equine	22	0	0	0	0%								
USA		Rabbit	3	0	0	0	0%								
USA		Ruminant	25	0	0	0	0%								
USA	Wastewater	Raw	13	5	38	12 (of 12)	100%								
USA		Final	12	5	42	8	67%								
Italy		Raw	41	20	49	NT	NT								
Italy		Final	41	16	39	NT	NT								
Italy	Surface water	Sea	12	0	0	NT	NT								
Italy		River	12	3	25	NT	NT								
USA		Fresh	15	0	0	0	0%								
USA	Groundwater	Raw	4	1	25	0	0%								
USA	Drinking water	Distribution	20	4	20	0 (of 11)	0%								

NT – not tested.

waters in the USA, but was detected in the majority of wastewater samples (100% of raw samples and 67% of treated samples).

## DISCUSSION

TTV is ubiquitous and has been reported in a wide range of fecal samples from warm-blooded animals and humans. It has also been detected in certain animal species, including non-human primates (Verschoor *et al.* 1999; Cong *et al.* 2000; Okamoto & Mayumi 2000), farm animals (pigs, chickens, cows, and sheep) (Leary *et al.* 1999; Devalle & Niel 2005; Sibila *et al.* 2009; Brassard *et al.* 2010; Martinez Guino *et al.* 2010; Lang *et al.* 2011; Liu *et al.* 2011), companion animals (dogs and cats) (Biagini *et al.* 2007; Okamoto 2009b; Zhu *et al.* 2011), and wild animals (wild boar and sea lions) (Martinez *et al.* 2006; Ng 2009). For example, a study of 158 fecal samples collected from dogs younger than 1 year old with diarrhea in a pet clinic in China showed that 20 specimens (20/158, 13%) were positive for Torque teno canis virus DNA using detection with PCR (Lan *et al.* 2011). While TTV has been identified in a variety of animal fecal samples, this study only had a 4.0% detection rate for TTV in fecal samples. In this study, a human based TTV sequence was utilized and therefore presence of this sequence would not be expected in animal fecal samples. Positive detection of human TTV in 4.0% of non-human animal feces may have been a result of human-animal cross infection. The one TTV positive chicken sample was from a private farm (and was weakly positive) and the two TTV positive dog samples were from companion animals.

TTV has been detected in feces and thus in sewage in prior research (e.g., Haramoto *et al.* 2008). The percentage of raw wastewater samples that are positive for TTV varies greatly in different studies: 97% in Japan (Haramoto *et al.* 2005), 50% in Germany (Hamza *et al.* 2011), and 13% in India (40 mL sample volume, Vaidya *et al.* 2002). In this study, quantitative data showed detectable levels of TTV in 100% ( $n = 11$ ) and 69% ( $n = 29$ ) of raw wastewaters from Australia and Italy, respectively. Using presence/absence data, 49% ( $n = 41$ ) and 38% ( $n = 13$ ) of raw wastewaters in Italy and the USA were positive for TTV, respectively. Although there were differences in samples volumes and analytical

methods, these values are comparable to previously published statistics. In this study, maximum concentrations of TTV were on the order of  $10^5$  genomic copies per mL in raw wastewaters in Italy and  $10^3$  genomic copies per mL in Australia. Results are comparable to Hamza *et al.* (2011), who found TTV at concentrations on the order of  $10^3$  genomic copies per mL in raw wastewater in Germany. Haramoto *et al.* (2008) detected TTV in 12 of 12 wastewater samples in Japan with a mean and maximum concentration of  $1.7 \times 10^4$  and  $4.8 \times 10^4$  genomic copies per liter (for comparison, on the order of  $10^7$  per mL), respectively. Removal rates of TTV through wastewater treatment in this study were similar to Hamza *et al.* (2011), who found 1.7–2.3 and 2.6–3.5  $\log_{10}$  removals for adenovirus and TTV, respectively. In India, TTV was isolated in 2% of wastewater effluent samples using 40 mL volumes. In this study, the percentage of treated wastewater samples with TTV (via quantitative or presence/absence testing) ranged from 39 to 100%, with differences based on sample volume, methodology, and treatment stage (before or after final disinfection).

The concentration or presence of viruses in environmental and drinking waters depends on the water source and influence of sewage discharges or other pollution sources on those waters. River water samples (10 L) collected from the Ruhr and Rhine Rivers in Germany in 2008–2009 were positive for adenoviruses in 108 of 111 (97.3%) of samples, and for TTV in 56 of 108 (51.9%) of samples (Hamza *et al.* 2011). These samples were collected in populated regions where wastewater treatment plant discharges were 1.5–9 km upstream of each sampling location. Diniz-Mendes *et al.* (2008) found high prevalence rates (92%) for TTV in samples from polluted streams in Brazil. In contrast, Vecchia *et al.* (2012) quantified TTV and fecal pollution in an urban area in Brazil that was influenced by non-treated sewage. TTV and adenovirus were found in 28.6% and 21.4% of river samples, respectively. A study of the Tamagawa River in Japan found TTV in only 5.6% of samples and adenovirus in 61.1% of samples (Haramoto *et al.* 2010). The sampling locations included a recreational area, and two sites with significant wastewater influences. In this study, samples were collected in water sources expected to be less influenced by wastewater discharges. While TTV and adenoviruses were found in all stormwater samples in Australia, median values were in the tenths to ones of genomic copies per mL. TTV was not found in



seawater samples in Italy, nor in surface waters in the USA. One groundwater sample was positive for TTV. Vecchia *et al.* (2012) found TTV below detection limits in surface waters in an area in Brazil with 92% sewerage, comparable to sewerage rates in areas of study in the USA.

## ANOVA

ANOVA was conducted on quantitative data sets to evaluate seasonal differences. There were no seasonal differences for indicators (bacteria or coliphages) in fecal samples, wastewaters, or fresh surface waters. For drinking water samples, seasonal differences were found for *E. coli* ( $n = 20$ ;  $p = 0.042$ ) and somatic coliphages ( $n = 20$ ;  $p = 0.016$ ). However, *E. coli* was below detection limits in all samples, and thus this variance was based on the detection limit rather than actual differences in samples. For somatic coliphages, only four of 20 samples were above detection limits. Blatchley *et al.* (2007) found that coliphages exhibited seasonal effects with concentrations higher in the summer than those observed in the winter in wastewaters. In this study, differences in phage concentrations were not seen in wastewaters (for which many more samples had concentrations above detection limits than in drinking waters).

In the literature, adenoviruses have been proposed as indicators of fecal pollution from human sources because of their culturability, resistance characteristics, and lack of seasonal variability (Jiang *et al.* 2001; Choi & Jiang 2005; Jiang *et al.* 2007; Simmons & Xagorarakis 2011). There were insufficient data in this study to test the seasonality of adenoviruses. An ANOVA was performed between countries for the wastewater samples, and somatic coliphages varied by country (USA and Italy;  $n = 107$ ;  $p < 0.001$ ). For TTV in wastewaters ( $n = 80$ ), there was no seasonal or geographical variation.

## Correlation analysis

Spearman Rank correlations were calculated for quantitative data sets and point biserial for binary data sets. Table 4 shows correlation analysis results for TTV in various matrices, though correlations were performed for all data sets with  $n \geq 20$  (full results not shown). In the fecal samples, there were correlations between the bacterial indicators and somatic coliphages, but no correlations to male-specific coliphages. TTV presence (of which only three samples were positive) was not correlated to any other parameter tested.

**Table 4** | Correlation analysis results (two tailed, 95%) for TTV versus other indicators and viruses. Statistically significant correlations in bold;  $p$ -value and  $n$  value in parentheses (ID = insufficient data if  $n < 20$ ; NA = data not collected)

Matrix	Parameter					
	Coliforms	<i>E. coli</i>	Enterococci	Male-specific coliphage	Somatic coliphage	Adenovirus presence
(a) TTV presence (point biserial correlations)						
Feces	0.156 (0.181, 75)	0.070 (0.553, 75)	NA	-0.025 (0.83, 75)	0.057 (0.624, 75)	a
Wastewater	0.050 (0.814, 25)	0.150 (0.123, 107)	<b>0.423 (0.001, 58)</b>	<b>0.421 (0.036, 25)</b>	0.138 (0.156, 107)	-0.302 (0.151, 24)
Surface water	ID	<b>0.396 (0.013, 39)</b>	NA	ID	<b>0.421 (0.008, 39)</b>	ID
Drinking water	-0.116 (0.627, 20)	-0.130 (0.584, 20)	NA	-0.115 (0.628, 20)	-0.177 (0.455, 20)	ID
Matrix	Parameter					
	<i>E. coli</i>	Enterococci	Somatic coliphage	Adenovirus (gc/mL)	Polyomavirus	Microviridae
(b) TTV (gc/mL) (Spearman correlations)						
Wastewater	<b>0.553 (0.000, 58)</b>	<b>0.524 (0.000, 58)</b>	<b>0.319 (0.015, 58)</b>	<b>0.710 (0.000, 22)</b>	<b>0.823 (0.000, 22)</b>	<b>0.740 (0.000, 22)</b>
Stormwater	0.102 (0.635, 24)	0.088 (0.683, 24)	-0.354 (0.089, 24)	-0.275 (0.193, 24)	0.144 (0.501, 24)	NA

<sup>a</sup>Cannot be computed because one variable constant.

In wastewater samples, expected correlations between bacterial indicators were found, and similarly, the quantitative virus data sets correlated to one another (TTV, adenovirus, polyomavirus, microviridae). In a similar study of sewage treatment, Vaidya *et al.* (2002) found TTV DNA correlated to enteric viruses in raw sewage where the prevalence of TTV DNA was statistically similar to the prevalence of hepatitis E virus RNA and hepatitis A virus RNA. This study also included TTV correlations to *E. coli*, enterococci, somatic coliphages, and viruses. TTV and microviridae (a family of bacteriophage which includes somatic phage  $\Phi$ X-174) may provide useful information based on their correlations to other viruses in wastewaters.

For the stormwater samples, relationships were identified between *E. coli*, enterococci and somatic coliphages, but very few correlations were found with other viral pathogens. In surface waters, TTV presence was correlated to *E. coli* and somatic coliphages; however, with very few samples positive for TTV, these relationships should be considered preliminary. Other data in the literature have shown no statistical correlation between somatic coliphages and enteroviruses, human adenovirus, or Norwalk (I and II) virus in rivers in France (Hot *et al.* 2003). In drinking waters, the number of TTV positive samples was again low; however in these samples, TTV was not correlated to other indicators.

## CONCLUSIONS

Currently, bacterial indicators such as coliforms, *E. coli*, and enterococci are applied to waters worldwide to indicate the potential risk for fecal contamination. The strengths and weaknesses of these indicators regarding this universal application are supported by over 100 years of use. Improvements in microbial diagnostics and disease surveillance have demonstrated that bacterial indicators are not always protective when the pathogens in question are viruses. Based on its characteristics and recent discovery, TTV has been investigated in multiple geographical locations for its potential as a universal indicator. This study analyzed three environmental monitoring data sets that included TTV from three different continents (North America, Europe, and Pacifica). These results were compared to those in the literature from Asia and South America. The results demonstrate the presence

and occurrence of TTV on all five continents; however, there was significant variability in environmental prevalence and concentrations. Comparisons of TTV to bacterial indicators and other viral indicators demonstrated that its occurrence and concentrations do not strongly correlate to either group. Thus, TTV monitoring could potentially provide supplemental information about a water's microbial content than bacterial indicators or other candidate viral indicators. However, the data sets were small and did not utilize the same methodologies. Overall, these results support the need for careful, coordinated investigation of TTV as a water quality viral indicator before it can be adopted or abandoned.

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## REFERENCES

- Abbaszadegan, M., Monteiro, P., Nwachuku, N., Alum, A. & Ryu, H. 2008 Removal of adenovirus, calicivirus, and bacteriophages by conventional drinking water treatment. *Journal of Environmental Science and Health Part A* **43** (2), 171–177.
- APHA, AWWA & WEF 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association, Washington, DC.
- Ashbolt, N. J., Grabow, W. O. K. & Snozzi, M. 2001 Indicators of microbial water quality. In: *Water Quality: Guidelines, Standards, and Health* (L. Fewtrell & J. Bartram, eds). World Health Organization, IWA Publishing, London, UK.
- Bae, J. & Schwab, K. J. 2008 Evaluation of murine norovirus, feline calicivirus, poliovirus, and MS2 as surrogates for human norovirus in a model of viral persistence in surface water and groundwater. *Applied and Environmental Microbiology* **74** (2), 477–484.
- Bassitt, L., Takei, K., Hoshino-Shimizu, S., Nishiya, A. S., Sabino, E. C., Bassitt, R. P., Focaccia, R., D'Amico, A., Chamone, D.

- F. & Ribeiro-dos-Santos, G. 2002 New prevalence estimate of TT virus (TTV) infection in low-and high-risk population from Sao Paulo, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* **44** (4), 233–234.
- Bendinelli, M., Pistello, M., Maggi, F., Fornai, C., Freer, G. & Vatteroni, M. L. 2001 Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans. *Clinical Microbiology Reviews* **14** (1), 98–113.
- Biagini, P., Gallian, P., Touinssi, M., Cantaloube, J. F., Zapitelli, J. P., de Lamballerie, X. & de Micco, P. 2000 High prevalence of TT virus infection in French blood donors revealed by the use of three PCR systems. *Transfusion* **40** (5), 590–595.
- Biagini, P., Uch, R., Belhouchet, M., Attoui, H., Cantaloube, J. F., Brisbarre, N. & de Micco, P. 2007 Circular genomes related to anelloviruses identified in human and animal samples by using a combined rolling-circle amplification/sequence-independent single primer amplification approach. *The Journal of General Virology* **88** (10), 2696–2701.
- Blatchley, E. R., Gong, W. L., Alleman, J. E., Rose, J. B., Huffman, D. E., Otaki, M. & Lisle, J. T. 2007 Effects of wastewater disinfection on waterborne bacteria and viruses. *Water Environment Research* **79** (1), 81–92.
- Bofill-Mas, S., Calgua, B., Clemente-Casares, P., La Rosa, G., Iaconelli, M., Muscillo, M., Rutjes, S., De Roda Husman, A. M., Grunert, A., Graber, I., Verani, M., Carducci, A., Calvo, M., Wyn-Jones, P. & Girones, R. 2010 Quantification of human adenoviruses in European recreational waters. *Food and Environmental Virology* **2**, 101–109.
- Brassard, J., Gagne, M. J., Houde, A., Poitras, E. & Ward, P. 2010 Development of a real-time TaqMan PCR assay for the detection of porcine and bovine Torque teno virus. *Journal of Applied Microbiology* **108** (6), 2191–2198.
- Carducci, A., Verani, M., Battistini, R., Pizzi, F., Rovini, E., Andreoli, E. & Casini, B. 2006 Epidemiological surveillance of human enteric viruses by monitoring of different environmental matrices. *Water Science and Technology* **54** (3), 239–244.
- Carducci, A., Morici, P., Pizzi, F., Battistini, R., Rovini, E. & Verani, M. 2008 Study of the viral removal efficiency in a urban wastewater treatment plant. *Water Science and Technology* **58** (4), 893–897.
- Carducci, A., Battistini, R., Rovini, E. & Verani, M. 2009 Viral removal by wastewater treatment: monitoring of indicators and pathogens. *Food and Environmental Virology* **1** (2), 85–91.
- Choi, S. & Jiang, S. C. 2005 Real-time PCR quantification of human adenoviruses in urban rivers indicates genome prevalence but low infectivity. *Applied and Environmental Microbiology* **71** (11), 7426–7433.
- Cong, M.-E., Nichols, B., Dou, X.-G., Spelbring, J. E., Krawczynski, K., Fields, H. A. & Khudyakov, Y. E. 2000 Related TT viruses in chimpanzees. *Virology* **274** (2), 343–355.
- Craun, G. F., Calderon, R. L. & Wade, T. J. 2006 Assessing waterborne risks: an introduction. *Journal of Water and Health* **4** (2), 3–18.
- Devalle, S. & Niel, C. 2005 A multiplex PCR assay able to simultaneously detect Torque teno virus isolates from phylogenetic groups 1 to 5. *Brazilian Journal of Medical and Biological Research* **38** (6), 853–860.
- Diniz-Mendes, L., Devalle, S. & Niel, C. 2004 Genomic characterization of a Brazilian TT virus isolate closely related to SEN virus-F. *Memórias do Instituto Oswaldo Cruz* **99** (3), 301–306.
- Diniz-Mendes, L., Paula, V. S. d., Luz, S. L. B. & Niel, C. 2008 High prevalence of human Torque teno virus in streams crossing the city of Manaus, Brazilian Amazon. *Journal of Applied Microbiology* **105**, 51–58.
- Hamza, I. A., Jurzik, L., Uberla, K. & Wilhelm, M. 2011 Evaluation of pepper mild mottle virus, human picobirnavirus and Torque teno virus as indicators of fecal contamination in river water. *Water Research* **45** (3), 1358–1368.
- Haramoto, E., Katayama, H., Oguma, K., Yamashita, H., Nakajima, E. & Ohgaki, S. 2005 One-year monthly monitoring of Torque teno virus (TTV) in wastewater treatment plants in Japan. *Water Research* **39** (10), 2008–2013.
- Haramoto, E., Katayama, H., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H. & Ohgaki, S. 2006 Seasonal profiles of human noroviruses and indicator bacteria in a wastewater treatment plant in Tokyo, Japan. *Water Science and Technology* **54** (11–12), 301–308.
- Haramoto, E., Katayama, H. & Ohgaki, S. 2008 Quantification and genotyping of torque teno virus at a wastewater treatment plant in Japan. *Applied and Environmental Microbiology* **74** (23), 7434–7436.
- Haramoto, E., Kitajima, M., Katayama, H. & Ohgaki, S. 2010 Real-time PCR detection of adenoviruses, polyomaviruses, and torque teno viruses in river water in Japan. *Water Research* **44** (6), 1747–1752.
- Hot, D., Legeay, O., Jacques, J., Gantzer, C., Caudrelier, Y., Guyard, K., Lange, M. & Andreoletti, L. 2003 Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water. *Water Research* **37** (19), 4703–4710.
- ISO 1998a ISO 9308–3. *Water Quality—Detection and Enumeration of Escherichia Coli and Coliform Bacteria. Part 3. Miniaturized Method (Most Probable Number) for the Detection and Enumeration of E. Coli in Surface and Waste Water*. International Organization for Standardization, Geneva.
- ISO 1998b ISO 7899–1. *Water Quality—Detection and Enumeration of Intestinal Enterococci. Part 1. Miniaturized Method (Most Probable Number) for Surface and Waste Water*. International Organization for Standardization, Geneva.
- ISO 1999 *Water Quality—Detection and Enumeration of Bacteriophages. Part 2: Enumeration of Somatic Coliphages*. International Organization for Standardization, Geneva, Switzerland.
- Jiang, S. C. & Chu, W. 2004 PCR Detection of pathogenic viruses in southern California urban rivers. *Journal of Applied Microbiology* **97** (1), 17–28.

- Jiang, S., Noble, R. & Chu, W. 2001 [Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California](#). *Applied and Environmental Microbiology* **67** (1), 179–184.
- Jiang, S. C., Chu, W. & He, J. W. 2007 [Seasonal detection of human viruses and coliphage in Newport Bay, California](#). *Applied and Environmental Microbiology* **73** (20), 6468–6474.
- Jothikumar, N., Cromeans, T. L., Hill, V. R., Lu, X., Sobsey, M. D. & Erdman, D. D. 2005 [Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41](#). *Applied and Environmental Microbiology* **71** (6), 3131–3136.
- Kopecka, H., Dubrou, S., Prevot, J., Marechal, J. & Lopez-Pila, J. M. 1993 [Detection of naturally occurring enteroviruses in waters by reverse transcription, polymerase chain reaction, and hybridization](#). *Applied and Environmental Microbiology* **59** (4), 1213–1219.
- Lan, D., Hua, X., Cui, L., Luo, X., Liu, Z., San, T., Zhu, C., Zhao, W. & Yang, Z. 2011 [Sequence analysis of a Torque teno canis virus isolated in China](#). *Virus Research* **160** (1–2), 98–101.
- Lang, C., Sollner, H., Barz, A., Ladinig, A., Langhoff, R., Weissenbock, H., Kekarainen, T., Segales, J. & Ritzmann, M. 2011 [Investigation of the prevalence of swine torque teno virus in Austria](#). *Berliner und Munchener Tierarztliche Wochenschrift* **124** (3–4), 142–147.
- Leary, T. P., Erker, J. C., Chalmers, M. L., Desai, S. M. & Mushahwar, I. K. 1999 [Improved detection systems for TT virus reveal high prevalence in humans, non-human primates and farm animals](#). *The Journal of General Virology* **80** (8), 2115–2120.
- Leclerc, H., Edberg, S., Pierzo, V. & Delattre, J. 2000 [Bacteriophages as indicators of enteric viruses and public health risk in groundwaters](#). *Journal of Applied Microbiology* **88** (1), 5–21.
- Lee, H. S. & Sobsey, M. D. 2011 [Survival of prototype strains of somatic coliphage families in environmental waters and when exposed to UV low-pressure monochromatic radiation or heat](#). *Water Research* **45** (12), 3723–3734.
- Liu, X., Gu, W., Guo, X., Ge, X., Chen, Y. & Yang, H. 2011 [Prevalence of torque teno virus infection in pig herds in China](#). *Veterinary Record* **168** (15), 410.
- Long, S. C., El-Khoury, S. S., Oudejans, S. J. G., Sobsey, M. D. & Vinje, J. 2005 [Assessment of sources and diversity of male-specific coliphages for source tracking](#). *Environmental Engineering Science* **22** (3), 367–377.
- Maggi, F. & Bendinelli, M. 2009 [Immunobiology of the Torque teno viruses and other anelloviruses](#). *Current Topics in Microbiology and Immunology* **331**, 65–90.
- Maggi, F., Fornai, C., Zaccaro, L., Morrica, A., Vatteroni, M. L., Isola, P., Marchi, S., Ricchiuti, A., Pistello, M. & Bendinelli, M. 2001 [TT Virus \(TTV\) loads associated with different peripheral blood cell types and evidence for TTV replication in activated mononuclear cells](#). *Journal of Medical Virology* **64** (2), 190–194.
- Maggi, F., Pifferi, M., Michelucci, A., Albani, M., Sbranti, S., Lanini, L., Simi, P., Macchia, P., Pistello, M. & Bendinelli, M. 2011 [Torque teno virus viremia load size in patients with selected congenital defects of innate immunity](#). *Clinical and Vaccine Immunology* **18** (4), 692–694.
- Martínez, L., Kekarainen, T., Sibila, M., Ruiz-Fons, F., Vidal, D., Gortázar, C. & Segalés, J. 2006 [Torque teno virus \(TTV\) is highly prevalent in the European wild boar \(\*Sus scrofa\*\)](#). *Veterinary Microbiology* **118**, 223–229.
- Martinez Guino, L., Kekarainen, T., Maldonado, J., Aramouni, M., Llorens, A. & Segalés, J. 2010 [Torque teno sus virus \(TTV\) detection in aborted and slaughterhouse collected foetuses](#). *Theriogenology* **74** (2), 277–281.
- McQuaig, S. M. & Noble, R. T. 2011 [Viruses as tracers of fecal contamination](#). In: *Microbial Source Tracking: Methods, Applications, and Case Studies* (C. Hagedorn, A. R. Blanch & V. J. Harwood, eds). Springer, New York, pp. 113–135.
- Muniesa, M. & Jofre, J. 2004 [Factors influencing the replication of somatic coliphages in the water environment](#). *Antonie Van Leeuwenhoek* **86** (1), 65–76.
- Ng, T. F. F. 2009 [Novel anellovirus discovered from a mortality event of captive California sea lions](#). *Journal of General Virology* **90** (5), 1256–1261.
- Nishizawa, T., Okamoto, H., Konishi, K., Yoshizawa, H., Miyakawa, Y. & Mayumi, M. 1997 [A novel DNA virus \(TTV\) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology](#). *Biochemical and Biophysical Research Communications* **241** (1), 92–97.
- Ogorzaly, L., Bertrand, I., Paris, M., Maul, A. & Gantzer, C. 2010 [Occurrence, survival, and persistence of human adenoviruses and F-specific RNA phages in raw groundwater](#). *Applied and Environmental Microbiology* **76** (24), 8019–8025.
- Okamoto, H. 2009a [History of discoveries and pathogenicity of TT viruses](#). *Current Topics in Microbiology and Immunology* **331**, 1–20.
- Okamoto, H. 2009b [TT Viruses in animals](#). *Current Topics in Microbiology and Immunology* **331**, 35–52.
- Okamoto, H. & Mayumi, M. 2000 [Molecular virology of TT virus \(TTV\)](#). *Virus* **50** (2), 259–271.
- Okoh, A. I., Sibanda, T. & Gusha, S. S. 2010 [Inadequately treated wastewater as a source of human enteric viruses in the environment](#). *International Journal of Environmental Research and Public Health* **7** (6), 2620–2637.
- Park, G. W. & Sobsey, M. D. 2011 [Simultaneous comparison of murine norovirus, feline calicivirus, coliphage MS2, and GII.4 norovirus to evaluate the efficacy of sodium hypochlorite against human norovirus on a fecally soiled stainless steel surface](#). *Foodborne Pathogens and Disease* **8** (9), 1005–1010.
- Payment, P. & Locas, A. 2011 [Pathogens in water: value and limits of correlation with microbial indicators](#). *Ground Water* **49** (1), 4–11.
- Payment, P., Trudel, M. & Plante, R. 1985 [Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants](#). *Applied and Environmental Microbiology* **49** (6), 1418–1428.

- Plummer, J. D. & Long, S. C. 2013 *Assessment of Torque Teno Virus as a Viral Pathogen Indicator in Waters (project 4288)*. Water Research Foundation, Denver, CO.
- Plummer, J. D., Long, S. C., Charest, A. J. & Roop, D. O. 2014 [Bacterial and viral indicators of fecal contamination in drinking water](#). *Journal of the American Water Works Association* **106** (4), E200–E211.
- Scott, T., Rose, J. D., Jenkins, T. M., Farrah, S. R. & Lukasik, J. 2002 [Microbial source tracking: current methodology and future directions](#). *Applied and Environmental Microbiology* **68**, 5796–5803.
- Shin, G. A. & Lee, J. K. 2010 [Inactivation of human adenovirus by sequential disinfection with an alternative ultraviolet technology and monochloramine](#). *Canadian Journal of Microbiology* **56** (7), 606–609.
- Sibila, M., Martinez-Guino, L., Huerta, E., Mora, M., Grau-Roma, L., Kekarainen, T. & Segales, J. 2009 [Torque teno virus \(TTV\) infection in sows and suckling piglets](#). *Veterinary Microbiology* **137** (3–4), 354–358.
- Sidhu, J. P. S., Hodggers, L., Ahmed, W., Chong, M. & Toze, S. 2012a [Prevalence of human pathogens and indicators in stormwater runoff in Brisbane, Australia](#). *Water Research* **46** (20), 6652–6660.
- Sidhu, J. P. S., Gernjak, W. & Toze, S. (Editors) 2012b *Health Risk Assessment of Urban Stormwater*. Urban Water Security Research Alliance Technical Report No. 102. CSIRO, Queensland, Australia. Available at: [www.urbanwateralliance.org.au/publications/UWSRA-tr102.pdf](http://www.urbanwateralliance.org.au/publications/UWSRA-tr102.pdf).
- Sidhu, J. P. S., Ahmed, W., Gernjak, W., Aryal, R., McCarthy, D., Palmer, A., Kolotelo, P. & Toze, S. 2013 [Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers](#). *Science of the Total Environment* **463**, 488–496.
- Simmons, F. J. & Xagorarakis, I. 2011 [Release of infectious human enteric viruses by full-scale wastewater utilities](#). *Water Research* **45** (12), 3590–3598.
- Toze, S., Hodggers, L., Mathews, B., Stratton, H., Ahmed, W., Collins, S., Schroeder, S. & Sidhu, J. 2012 *Presence and Removal of Enteric Microorganisms in South East Queensland Wastewater Treatment Plants*. Urban Water Security Research Alliance Technical Report No. 55. CSIRO, Queensland, Australia. Available at: <http://www.urbanwateralliance.org.au/publications/UWSRA-tr55.pdf>.
- US EPA 2001 *Method 1602: Detection of Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure*. US EPA Office of Water, Washington, DC (EPA 821-R-01-029).
- Vaidya, S. R., Chitambar, S. D. & Arankalle, V. A. 2002 [Polymerase chain reaction-based prevalence of hepatitis A, hepatitis E and TT viruses in sewage from an endemic area](#). *Journal of Hepatology* **37** (1), 131–136.
- Vecchia, A. D. 2009 [Torque teno virus \(TTV\) and fecal pollution](#). *Revista de Patologia Tropical* **38** (3), 145.
- Vecchia, A. D., Fleck, J. D., Comerlato, J., Kluge, M., Bergamaschi, B., Da Silva, J. V. S., Da Luz, R. B., Teixeira, T. F., Garbinatto, G. N., Oliveira, D. V., Zanin, J. G., Van der Sand, S., Frazzon, A. P. G., Franco, A. C., Roehe, P. M. & Spilki, F. R. 2012 [First description of adenovirus, enterovirus, rotavirus and Torque teno virus in water samples collected from the Arroio Dilúvio, Porto Alegre, Brazil](#). *Brazilian Journal of Biology* **72** (2), 323–329.
- Verani, M., Casini, B., Battistini, R., Pizzi, F., Rovini, E. & Carducci, A. 2006 [One-year monthly monitoring of Torque teno virus \(TTV\) in river water in Italy](#). *Water Science and Technology* **54** (3), 191–195.
- Verschoor, E. J., Langenhuijzen, S. & Heeney, J. L. 1999 [TT Viruses \(TTV\) of non-human primates and their relationship to the human TTV genotypes](#). *Journal of General Virology* **80** (9), 2491–2499.
- Westrell, T., Teunis, P., van den Berg, H., Lodder, W., Ketelaars, H., Stenström, T. A. & de Roda Husman, A. M. 2006 [Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period](#). *Water Research* **40** (14), 2613–2620.
- Wu, J., Long, S. C., Das, D. & Dorner, S. 2011 [Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research](#). *Journal of Water and Health* **9** (2), 265–278.
- Zhu, C. X., Shan, T. L., Cui, L., Luo, X. N., Liu, Z. J., Tang, S. D., Liu, Z. W., Yuan, C. L., Lan, D. L., Zhao, W. & Hua, X. G. 2011 [Molecular detection and sequence analysis of feline Torque teno virus \(TTV\) in China](#). *Virus Research* **156** (1–2), 13–16.

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