Comments on the US EPA "Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality"

Prepared for the National Association of Clean Water Agencies (NACWA)

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Executive Summary

Millions of people each year enjoy using beaches, lakes, and rivers for recreation. Disease-causing microbes – pathogens – found in surface waters can present a threat to public health, particularly as a cause of gastrointestinal illness. Viral pathogens have been difficult, costly, and time-consuming to measure in surface waters. In order to address the challenges of 1) estimating the likelihood of pathogen presence and pathogen concentration in surface waters, and 2) estimating the health risks of surface water recreation, "indicators" have been monitored in surface waters as an alternative to pathogens. For decades, fecal indicator bacteria, such as *E. coli* and enterococci bacteria have been monitored in surface waters to satisfy a variety of Clean Water Act requirements.

Coliphage viruses – viruses that infect *E. coli* bacteria – have been evaluated as indicators of wastewater treatment efficacy, human fecal pollution of surface waters, pathogenic virus presence in surface waters, and human health risk. In April, 2015, the US EPA Office of Water published a review of coliphage virus as a potential indicator of pathogens in surface wasters. That publication, "Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality," included reviews of research studies that evaluated coliphage measurements as predictors of health risks of water recreation and pathogen presence. The present document, prepared for the National Association of Clean Water Agencies (NACWA), provides information from research papers that were not included in the EPA review as well as additional information from papers that were included in the EPA review. The "charge" questions of NACWA are listed on page 7 of this document, address relationships between coliphage and viral pathogens in recreational waters, and coliphage as a predictor of illness among people who use recreational surface waters for recreation.

Key findings of this review of studies that evaluated coliphages as predictor of viral pathogen presence in surface waters are:

- The methods used to concentrate and test water samples for viral pathogens varied substantially across studies, in part because such methods have changed over the past 20 years.
- The statistical methods used to analyze associations between coliphages and viral pathogens were often incompletely described, and some studies did not seem optimal for the types of data that were collected.
- None of the studies reviewed described crucial performance characteristics of coliphages as predictors of pathogen presence, namely the sensitivity, specificity, positive predictive value and negative predictive value of coliphages.
- Findings of the coliphage and viral pathogen literature reviewed demonstrated inverse associations (high coliphage concentrations makes pathogen absence more likely), direct associations (high coliphage concentrations makes pathogen presence more likely), and in many cases, no association.

- These conflicting results may be due in part to the variability in laboratory and data analysis methods across studies, the relatively few water samples analyzed in many studies, and the differing proximity to a variety of fecal pollution sources across studies.

Key findings of this review of studies that evaluated coliphages as predictor of illness among water recreators are:

- The available scientific literature regarding coliphages and health risks of water recreation – eight published studies - is quite limited.
- The epidemiologic studies that evaluated coliphages arrived at conflicting conclusions about the predictive value of these viruses as predictors of health risk following surface water recreation.
- Relatively few swimmers have been enrolled into studies of coliphages as predictors of health risk compared to the number of swimmers enrolled into EPA's epidemiologic studies that have been used to develop water quality criteria and/or beach action values.

Regarding both potential uses of coliphages (as a surrogate for infectious enteric viral pathogens and as a predictor of the risk of illness among water recreators):

- A substantial amount of additional research is needed before coliphage testing could be recommended with confidence in surface water monitoring frameworks.

- In order to characterize coliphage concentrations as predictors of the presence or concentration of infectious viral pathogens, multi-site studies of sufficient size are needed. The waters sampled would have varying fecal pollutant sources and different hydrologic characteristics. Protocols for coliphage and infectious viral pathogen testing would optimized and then performed in a variety of laboratories. Basic performance characteristics of sensitivity, specificity, positive predictive value, negative predictive value, measures of association such as the increase in probability of detecting infectious viral pathogens for a given change in coliphage concentration.

- Additional and larger epidemiologic studies conducted in fresh and marine waters, settings would be needed in order to evaluate the predictive value of coliphage testing and whether such testing adds to the predictive value of information generated through the monitoring of fecal indicator bacteria.

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List of abbreviations

BGM cell culture	Buffalo green monkey kidney cell culture
CFU	colony forming unit
CLAT	F+ coliphage detection
CLAT	coliphage latex-agglutination test
Ct	cycle threshold
FIB	fecal indicator bacteria
GI	Gastrointestinal
GM	geometric mean
HCGI-1	Highly credible gastrointestinal illness, definition 1
ICC n-PCR	integrated cell culture with nested PCR detection
MPN	most probable number
NACWA	National Association of Clean Water Agencies
NEEAR	National Epidemiological and Environmental Assessment of Recreational
NOAEL	no observed adverse effect level
OR	odds ratio
PDU	PCR-detectable units
PFU	plaque forming unit
POTWs	publicly owned treatment works
qPCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
ROC	receiver operating characteristic
RR	relative risk
RT-PCR	real time PCR, reverse transcription PCR
SC	somatic coliphage
TCV-MPN	Total culturable virus most probable number
US EPA	US Environmental Protection Agency
WERF	Water Environment Research Foundation
WQ	water quality

Background

In the US dozens of outbreaks of disease linked to recreation at lakes and rivers occur annually [1-3]. Bacteria, viruses, and protozoa have been identified as etiologic agents responsible for these recognized outbreaks. Outside of the context of outbreaks, sporadic cases of illness attributable to water recreation occur with some frequency at US surface waters – approximately 15-25 per 1,000 water recreators [4-6]. The etiologic agents responsible for these sporadic cases of illnesses have not been identified by epidemiologic investigations [7, 8].

In order to protect the health of the public, the US Environmental Protection Agency (EPA) has conducted epidemiologic studies of water recreation, in which participants are enrolled at beaches, beach water is tested for microbes, and water quality is used to predict risk of illness among swimmers. Based on such studies, EPA has developed "criteria" that describe microbes to be measured, the frequency for measuring those microbes, and the values of those microbes that indicate an elevated health risk. Based on studies conducted in the late 1970s and early 1980s, in 1986 EPA published criteria values for freshwater beaches (*E. coli* and enterococci measured by culture) and marine beaches (enterococci measured by culture) [9]. Based on epidemiologic studies conducted in the past decade, EPA published updated Criteria [10]. Those Criteria, while similar in many ways to 1986 Criteria, described a method, the quantitative polymerase chain reaction (qPCR), for evaluating water quality that generates same-day results, which can be useful for timely public notification of water quality at beaches. In addition to their use in beach monitoring and notification programs, water quality Criteria are also used for a variety of other Clean Water Act purposes. These include the establishment of "Total Maximum Daily Loads" and the designation of water bodies as "impaired."

Several epidemiologic studies in the past 25 years have evaluated coliphage viruses – viruses that infect *E. coli* bacteria – as a predictor of health risk among swimmers. Many more studies evaluated coliphages as predictors of viral pathogen presence or concentration in surface waters. In 2015 the EPA Office of Water published a review of the environmental health literature to evaluate "… the potential for coliphages to be useful as viral indicators of fecal contamination." Findings of the literature summary and critique were published as "Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality" [11], which is referred to in the present report as "the EPA review."

To complement findings of the EPA review, the National Association of Clean Water Agencies (NAWCA) sought to further characterize associations between 1) coliphage and health outcomes following surface water recreation, and 2) coliphages and viral pathogens in surface waters. This report summarizes a review of studies cited in EPA's coliphage review, as well as additional relevant studies, some of which were published after the EPA Review was written. This review is funded by the National Association of Clean Water Agencies (NACWA) and the Water Environment Research Foundation (WERF) to address the following questions:

1. Is there a relationship between male specific and/or somatic coliphage with enteric viruses in recreational waters? If so, what is that relationship (presence/absence, dose- response, etc.)? Recreational waters are those designated for this specific use in state or federal water quality standards regulations. For example, a storm water channel is not normally designated for recreational use, but a lake with a beach would be designated for this use.

2. Is there a relationship between male specific and/or somatic coliphage with human health in recreational waters? If so, what is that relationship?

3. Is there a relationship between enteric viruses and human health in recreational waters? If so, what is that relationship?

4. Do any of these papers link coliphage or viruses originating from wastewater that is discharged by centralized facilities to human health? If so, what is the nature of this link and what are the circumstances characterizing the link?

5. Are there other recreational water studies not referenced by EPA that evaluate each of the relationships above and meet current conventional standards for epidemiological study? Do these studies change the response to the questions above, and if so, how and why?

Efforts to answer questions 2-5 (which all address human health outcomes) were considered together.

Terminology used in the coliphage and environmental health literature varies. In order to avoid confusion in comparing studies, some terms used in this review differed from terms used in the original studies. Some studies use the abbreviation RT-PCR to refer to "real time PCR" while others use it to mean 'reverse transcription PCR.' To avoid that confusion, the abbreviation RT-PCR is not used in this report, even if it had been used in the studies reviewed. Reverse transcription PCR that is used in presence/absence tests of RNA viruses is referred to as "endpoint PCR" here. The term "qPCR" (quantitative PCR) is used here rather than "real-time PCR." In this report coliphages are referred to as F+ coliphage (rather than "male-specific") or somatic coliphage (rather than "F-coliphage" or "F minus coliphage"). Measures of microbes per volume of water are referred to as "concentration" even if the original study used the term "density."

Relationship between coliphages and enteric viral pathogens in surface waters

In order to answer charge question 1 (relationship between coliphage and enteric viruses), a total of 19 publications were reviewed, including several which were not included in the EPA review (in some cases because they were published after the review was conducted). The primary literature (rather than review articles) was summarized in three ways. A "10,000 foot view," a general summary of major elements of the design and findings of studies is found in Level One: Overall summary . More information about major elements of individual studies are described in Level Two: Brief summaries of studies of coliphage and viral pathogens in surface water, while additional details of study methods and findings are provided in Level Three: Coliphage and viral pathogen literature summary.

Level One: Overall summary

The studies summarized in Table 1 varied substantially in terms of study objectives, as some sought primarily to address questions regarding seasonal variability in microbe concentrations or viral persistence in surface waters, rather than the associations between coliphages and viral pathogens. In such cases, few details were provided that would be of primary interest to this review. Other sources of variability among studies include the number of water samples analyzed, water sampling methods, virus concentration methods, limits of detection and quantification, virus detection methods, and data analysis methods. Importantly, testing water samples for enteric viruses using culture methods (alone or followed by PCR), can identify infectious viruses. Methods that use PCR only do not differentiate between infectious viruses and non-viable viruses (or viral nucleic acids [DNA or RNA] that cannot by themselves cause infection). For that reason, inferences regarding health risk should be drawn with caution from studies of coliphage-pathogen association that do not use enteric viral pathogen culture methods.

Statistical methods for testing hypotheses regarding associations between coliphages and viral pathogens were not well described in several studies, and in others, did not seem optimal for the types of data (presence/absence, ordinal categories, or measured concentrations) or the distributions (normal, log-normal, non-normal) of the data. It is fair to say that the authors of the primary research studies did not have this review in mind when they designed, conducted, and described their study results. Ideally, studies that characterize associations between viral pathogens presence and a continuous measure of coliphages would describe results of logistic regression models or receiver operator characteristics, but only one study [12] did that. The use of categories of coliphage values (above vs. below a threshold value or even presence/absence) to predict viral pathogen presence would note the rates of true positives (coliphage above a threshold concentration value, viral pathogen present), false positives (coliphage above that threshold, viral pathogen absent), true negatives (coliphage below a threshold value, viral pathogen not detected), and false negatives (coliphage below a threshold value, viral pathogen present). With such information, the coliphage method could be evaluated in terms of sensitivity, specificity, positive predictive value, and negative predictive value. None of the studies reported this basic set of characteristics of a screening test. The degree to which coliphage and viral pathogen data agree with one another could be described in other ways, such as

odds ratios, goodness of fit, and correlation coefficients, but few studies provided this type of information. Others simply noted p-values, indicating that the relationship between coliphage and viral pathogen data is unlikely due to chance, without providing correlation coefficients (and their confidence intervals) or other pieces of information that describe the relationship.

As summarized in Table 1, studies generated conflicting results as to whether statistically significant associations are found in surface waters. Some studies found no association, others found strong associations, and several reported inverse associations (viral pathogen detection is more likely with decreasing coliphage concentration). Several studies that evaluated more than one viral pathogen found associations between one pathogen and coliphage, but not with others. A total of three large studies listed in Table 1 utilized enteric viral pathogen culture methods to determine viral pathogen presence or concentration. Of these, only one identified culturable viral pathogens in water samples, and that one did find statistically significant associations between coliphages and some viral pathogens [13].

In summary, it appears that coliphages may have value as predictors of waterborne enteric viral pathogens. However, the studies, which were generally small and provided limited methodologic information about data analysis, generated contradictory results. Studies are needed to characterize the sensitivity, specificity, predictive value, and threshold values of coliphage that suggest the likely presence of infectious viral pathogens. Such studies would ideally be conducted collaboratively at multiple laboratories to promote consistency and optimization of methods, as was done in the development of the qPCR method for water quality monitoring. Ideally, such studies would use methods with low detection limits for infectious enteric viruses. The information currently available is presently insufficient to recommend with confidence the use coliphage as a surrogate for infectious enteric virus testing or for routine water quality monitoring.

Study	Wastewater impacted?	Study size*	Pathogen analyzed by culture?	Indicators as predictors of viral pathogen(s)	
Griffin, 1999 [14]	Septic systems	Small	No	SC and several viral pathogens	No
Jiang, 2001 [15]	Not described	Small	No	F+ coliphage and adenovirus	Yes
				FIB and adenovirus	No
Hot, 2003 [16]	Not directly	Medium	Yes	SC and culturable enterovirus	No
			No	SC and enterovirus RNA	No
Jiang, 2004 [17]	Yes (disinfected)	Small	No	SC and several viruses	No
				FIB and viruses	No
Skraber, 2004 [18]	Yes	Large	Yes	No enterovirus cultured	-
			No	SC and enteric virus	Yes
				FIB and enteric virus	Yes
Ballester, 2005 [19]	Yes	Large	Yes	SC and enteric virus, adenovirus	Yes
				SC and enterovirus, rotavirus	No
				F+ and enteric virus, rotavirus adenovirus	Yes
				F+ and astrovirus	No
				FIB and virus: no FIB quantifiable	-
Choi 2005 [20]	Some sites	Large	Yes	Adenovirus: all cultures negative	-
			No	F+, SC and adenovirus, enterovirus	No
			No	FIB and adenovirus, enterovirus	No
Moce´-Llivina, 2005 [21]	Yes	Small	Yes	SC and enterovirus	Yes
		Small	Yes	FIB and enterovirus	Yes
Jiang, 2007 [22]	No	Large	No	F+ and enterovirus, adenovirus	No
				FIB and enterovirus, adenovirus	No
Boehm, 2009 [23]	No	Medium	No	SC, F+ and enterovirus	No
				FIB and enterovirus	Inverse

(This table is continued on the following page)

SC: Somatic coliphage; F+: F+ coliphage; FIB: one or more fecal indicator bacteria, such as fecal coliforms, *E. coli*, or enterococci

*Study size defined by number of samples tested for both coliphage and viral pathogens: Small, <25 samples; medium, 25-74 samples; large ≥75

Study	Wastewater impacted?	Study size*	Pathogen analyzed by culture?	Indicators as predictors of viral pathogen(s)	
Espinosa, 2009 [24]	No	Large	No	Coliphage and adenovirus	No
				Coliphage and enterovirus	Yes
				Coliphage and astrovirus	No
Jurzik, 2010 [25]	Yes	Large	No	SC and adenovirus, norovirus, rotavirus	No
Lodder, 2010 [26]	Yes	Medium[a]	Yes	F+, SC and enterovirus, reovirus	Yes
			No	F+, SC and norovirus, rotavirus	No
Haramoto, 2011 [27]	No	Small	No	F+ and viral, protozoan pathogens	No
				FIB and viral, protozoan pathogens	Yes
Viau, 2011 [28]	No point source	Large	No	F+, SC and adenovirus, norovirus, enterovirus	No
				FIB and norovirus, adenovirus	Inverse
Love, 2014 [29]: Avalon beach	No point source	Large	No	F+ and adenovirus	No
				FIB and adenovirus	Yes
Love, 2014 [29]: Doheny beach	No point source	Large	No	F+ and adenovirus	Inverse
				F+, SC and norovirus	No
				SC and adenovirus	No
				FIB and adenovirus	Inverse
				FIB and norovirus	No
Rezaeinejad, 2014 [30]	No	Medium	No	F+, SC and norovirus	Yes
Liang, 2015 [31]	No	Large	No	F+, SC and norovirus, adenovirus	No
				FIB and norovirus, adenovirus	Yes
Updyke, 2015 [32]	Some sites	Medium	Enterovirus: yes, if PCR +	F+ RNA coliphage and enteric virus	No
				FIB and enteric viruses	No

Table 1 (continued) Summary of findings of studies that address coliphage-viral pathogen associations in surface waters

(This table is continued from the preceding page)

SC: Somatic coliphage; F+: F+ coliphage; FIB: one or more fecal indicator bacteria, such as fecal coliforms, *E. coli*, or enterococci

*Study size defined by number of samples tested for both coliphage and viral pathogens: Small, <25 samples; medium, 25-74 samples; large ≥75

[a]Although 75 samples were collected, only 69 were analyzed for all viral pathogens.

Level Two: Brief summaries of studies of coliphage and viral pathogens in surface water

Griffin, 1999 (not included in EPA review, Table 8)

In this study, 17 water samples were collected in the Florida Keys canals and two samples were collected from beach sites. Nearly all canal sites were near homes that used a septic system. Samples were analyzed by PCR (not culture) for enteroviruses, as well as hepatitis A, norovirus, and small round structured viruses. Measures of association were not reported, however, somatic coliphage was detected in 2 of 19 samples. These two samples were both positive for hepatitis A virus (present in 12 of 19 samples), demonstrating a significant problem with "false negatives".

Jiang, 2001

One water sample was collected at the mouth of each of 12 creeks in Southern California at the point at which the creeks flow into the Pacific Ocean. Pollutant sources were not described. Human adenovirus DNA was measured by PCR. Despite the small number of samples, an extremely strong correlation was found between F+ coliphage and human adenovirus (r=0.99). A correlation between "general" coliphage (F+ and somatic) and human adenovirus was suggested (r=0.32) but did reach statistical significance. The exceedance of threshold values for fecal indicator bacteria was not associated with human adenovirus presence.

Hot, 2003

A total of 68 samples collected from four French rivers were analyzed. The rivers receive wastewater discharges, but the samples were collected upstream of outfalls. Culturable enterovirus was rarely detected (2 of 68 water samples). The presence vs. the absence of enterovirus was not associated with somatic coliphage concentrations. Enterovirus RNA was frequently detected (60 of 68 water samples) and the presence of enterovirus RNA was not associated with concentrations of somatic coliphage.

Jiang, 2004 (not included in EPA review, Table 8)

A total of 21 samples were collected from urban rivers in the Los Angeles, California area, some of which were impacted by tertiary treated wastewater. Enterovirus, adenovirus, and hepatitis A virus presence, were determined by PCR or nested PCR, but not by viral culture. Although the each pathogenic virus was detected in 52-76% of samples, virus presence was not associated with somatic coliphage or fecal indicator bacteria (that data analysis was not included in the manuscript but logistic regression analysis was done to supplement this literature review using data presented in the paper).

Skraber, 2004

In this study of 90 water samples collected from the Moselle River in France from five sites of varying distances from urban wastewater discharges, enterovirus was measured by culture, and both enterovirus and norovirus genogroup II were measured by endpoint PCR. No culturable enterovirus was identified, though genomes of both viral pathogens were detected. Graphs in the paper demonstrate

clear associations between the frequency of detecting genetic material from the pathogenic viruses as categories of somatic coliphage concentration, though statistical testing of these associations was not reported.

Ballester, 2005

In this large 5-year study (the number of samples was not spelled out), water was collected from 5 points in the Massachusetts Bay, which receives treated wastewater through an outfall. Coliphage analysis methods changed after the second year of the study. Viral pathogens were quantified using integrated cell culture with nested PCR detection (ICC n-PCR). The ICC n-PCR results were analyzed as presence/absence data and concentrations of enteric viral pathogens were not described. Somatic coliphage was associated with enteric virus and adenovirus detection, but not with astrovirus, rotavirus or enterovirus. F+ coliphage was associated with the presence of all viral pathogens except for astrovirus. All indicator bacteria samples were reported as "below statistical counts <30 CFU/plate)". Spatial information (differences in detection frequency or concentration of viral pathogens in relation to the outfall diffuser) was presented descriptively, rather than quantitatively. However, it seems that proximity to and direction from the diffuser head may have differential impacts on the detection of different viruses.

Choi, 2005 (not included in EPA review, Table 8)

In this study, at total of 114 water samples from two urban rivers in Southern California (one of which received tertiary treated wastewater) were analyzed for human adenovirus (by culture and PCR) and enterovirus. No statistically significant correlations were observed between either viral pathogen and coliphages or fecal indicator bacteria.

Moce´-Llivina, 2005

In this study, 20 water samples from beaches in Barcelona, Spain, were analyzed. The beaches were impacted by wastewater from an underwater outfall and from rivers that carry secondary treated effluent. Seawater samples were analyzed by PCR for enterovirus; enterovirus cultures were also performed, with subsequent PCR. Relatively little detail is available about methods for evaluating association, but receiver operating characteristic testing demonstrate that concentrations of somatic coliphages and concentrations of enterococci were predictive of enterovirus.

Jiang, 2007

In this study, 206 samples collected from rivers that flow into Newport Bay, California, and from beaches on the Bay were analyzed. Point sources of fecal pollution were not described, but the Bay receives runoff from urban and agricultural areas. Enterovirus and adenovirus detection was infrequent (<5% of samples) and their presence was not associated with F+ coliphage or fecal indicator bacteria.

Boehm, 2009

In this study, water samples were collected once per hour for 72 hours from Avalon Beach, California, which is impacted by leaky sewage pipes. The primary focus on this research was the photoinactivation of pathogens and indicator microbes. No adenovirus was detected by endpoint PCR, but enterovirus was detected. No statistically significant correlations were observed between enterovirus and either F+ or somatic coliphage. Statistically significant inverse associations were observed between enterovirus and FIB (*E. coli* and enterococci).

Espinosa, 2009

In this study 80 samples were collected from a system of irrigation canals and from drinking water wells near Mexico City, Mexico. The canals may have had non-point sources of fecal pollution, but wastewater discharges were not mentioned. Coliphages were analyzed by culture, but the study did not state whether it was F+ or somatic coliphage (or both). Enteric viruses were measured by endpoint PCR (without culture). Coliphages found to be associated with enterovirus (p-value: 0.0182), but not with rotavirus or astrovirus (p-values: 0.150 and 0.459).

Jurzik, 2010 (not included in EPA review, Table 8)

This study involved the analysis of 190 samples collected from four locations on the Ruhr River in Germany that were impacted by wastewater. Viral pathogens were measured by PCR, not by culture. Numerous tests of association between somatic coliphage and a viral pathogen RNA (including adenovirus, norovirus, and rotavirus) were reported. None of these were statistically significant at a p=0.05 level, though somatic coliphage was associated with polyomavirus (not thought to be a cause of gastroenteritis in humans, though lifelong asymptomatic infection is thought to be common) only in waters with the temperature above 10 °C. Several statistically significant associations between fecal indicator bacteria and viral pathogen RNA (*E. coli* and rotavirus, coliforms and rotavirus) were observed, as well as associations between fecal indicator bacteria and polyomavirus.

Lodder, 2010

This analysis of 75 water samples collected at ten locations in the Netherlands impacted by wastewater discharge found that coliphage was correlated with enterovirus (measured by culture) but not with

reovirus (measured by culture), norovirus or rotavirus (both measured by PCR). The correlation itself was not described by a correlation coefficient value, only a p-value. The coliphage method a high rate of "false negatives," as in the two samples that tested negative for F+ coliphage, infectious enterovirus was present, and in one of those two samples, norovirus and rotavirus were also present.

Haramoto, 2011 (not included in EPA review, Table 8)

This analysis involved nine water samples from shallow groundwater wells and one sample from a polluted river in the Kathmandu Valley, Nepal. Nucleic acid from two viral pathogens (norovirus and adenovirus) were measured by qPCR (not culture). Two protozoan pathogens (Giardia and Cryptosporidium) were also analyzed in water samples. Association between pathogen presence and coliphage presence (or concentration) were not reported. However, of the six samples that did not contain F+ coliphage, two contained pathogens. The presence of a pathogen was more likely in water samples in which *E. coli* was detected, and this was reported to be statistically significant.

Viau, 2011

This analysis involved testing of 88 water samples from 22 Hawaiian streams for coliphage, viral pathogens, bacterial pathogens, bacterial indicators, and microbial source tracking markers. The waters sampled did not receive wastewater discharge. No associations were identified between coliphages and enterovirus or adenovirus. Adenovirus detection was inversely association with *E. coli* concentrations; adenovirus and norovirus genogroup I were inversely associated with a human-specific Bacteroides marker.

Love, 2014

This relatively large study was conducted at two beaches not thought to be impacted by wastewater discharge. However, norovirus RNA was found in 22.3% of samples at one of the beaches (Doheny), indicating substantial human fecal pollution. At the beach with more frequent viral pathogen detection (Doheny), adenovirus detection was inversely associated with measures of F+ coliphage and of enterococci. Adenovirus detection was directly associated with measures of fecal coliforms (finding high fecal coliforms concentrations makes adenovirus presence more likely). Somatic coliforms were not predictive of adenovirus. Norovirus presence could not be predicted by the coliphages or fecal indicator bacteria.

At Avalon beach, which had less frequent detection of adenovirus DNA (9.3% of samples, compared to 25.5% at Doheny beach), higher fecal coliform and enterococci concentrations were associated with a greater probability of detecting adenovirus. Higher F+ coliphage concentrations was suggestive of a greater probability of detecting adenovirus DNA, but this was of borderline statistical significance ($p=0.1$).

Liang, 2015 (not included in EPA review, Table 8)

This analysis involved 148 water samples from a stormwater reservoir and also from rivers and canals in Singapore. Viral pathogens were measured using qPCR, not culture. Although F+ and somatic coliphages were detected in over 90% of all samples, and rotavirus and norovirus genogroup II were detected in 48% and 39% of samples, there was no significant correlation between the coliphages and the viral pathogens (coliphage was associated with two pathogenic bacteria, Salmonella and Pseudomonas). In contrast to these findings, the fecal indicator bacteria were significant predictors of viral pathogen presence and viral pathogen nucleic acid concentration. The authors suggest that this may be due to contamination from non-human sources and the fact that they used a plaque assay method instead of genotyping of the male-specific RNA coliphages using RT-qPCR.

Updyke 2015

Samples were collected from 18 sites in Hawaii on six occasions. Some sites were near sewage treatment facilities. Samples were tested for FIB and for F+ RNA coliphages by culture. Enteric viral pathogens presence was evaluated by PCR, and samples positive for enterovirus were cultured to evaluate enterovirus infectivity. No samples that were positive for enterovirus on PCR testing showed infectivity on culture. No significant associations between enteric viruses and fecal indicator bacteria were found. Whether coliphage-enteric virus associations were found was not reported but data in table 3 indicates no association.

Not included in this review

In addition to the above studies several other publications were identified that described both coliphage and viral enteric pathogen presence or concentration in surface water [33, 34]. However, not enough information was provided to evaluate associations between coliphage and viral pathogens.

Several studies were not included in this review, but included in the EPA review. These are:

1. A study by Baggi et al. [35] focused on changes in virus concentration through the wastewater treatment process. Data in the paper do not allow evaluations of associations between coliphages and viral pathogens in the receiving waters.
2. A study by Betancourt and Rose [36], which did not contain quantitative information about pathogenic virus presence.
3. A study by Westrell et al. [37] did not include measures of association between coliphages and viral pathogens.

4. A consolidation of three prior studies by Payment and Locas [38] only included data that would only support analysis of potential associations between coliphages and viral pathogens in groundwater

Level Three: Coliphage and viral pathogen literature summary

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Griffin, 1999 **Setting** The Florida Keys 17 samples were taken from canal sites and 2 from nearshore water sites. **Fecal pollution sources** Although no sources of contamination are explicitly mentioned, the canal sites have been mentioned by the USEPA as being suspected of poor quality and on most canals use septic systems.	**Coliphages and analysis methods** Culture on *E. coli* ATCC 15597 used to determine "non-specific" (RNA and DNA somatic coliphage) concentrations. Culture on *E. coli* Famp to culture and then genotype F+ RNA coliphage. **Detection** Non-specific coliphages: 10 PFU/100mL in both cases where they were found. F+RNA coliphages not detected at any site.	**Viral pathogens and analysis methods** The presence of poliovirus, coxsackie A and B viruses, echovirus, hepatitis A, Norwalk viruses (norovirus) and small round-structured viruses was determined by endpoint PCR. Viral culture of pathogenic viruses was not done. **Viral pathogen detection** 79% of samples were positive when assayed with the pan-enterovirus primer set. 63% were positive for hepatitis A	19 samples were analyzed. Measures of association were not reported. However, somatic coliphage was detected in 2 of 19 samples. These two samples did not stand out in terms of viral pathogens detection.	Measures of association were not reported.

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Jiang, 2001 **Setting** Samples collected from 12 Southern California river and creeks at point where freshwater flows into the Pacific ocean. No explicit mention of pollution or sources.	**Coliphage types and measurement methods** A two agar layer method was utilized to detect coliphages. *E. coli* ATCC 15597 host for DNA and RNA coliphages (this includes somatic and F+ coliphages) *E. coli* HS (pFamp)R host for F+ coliphage **Coliphage presence** "Coliphage" present in 12 of 12 samples. F+ coliphage quantifiable in 5 of 12 samples. **Coliphage concentration** Mean "coliphage" concentration (average calculated by hand): 390.17 PFU/ liter Mean F+ coliphage concentration: 74.14 PFU/ liter	**Viral pathogen detection method** Nested PCR (without viral culture) **Viral pathogen nucleic acid detection** Human adenovirus DNA detected in 4 of 12 samples **Viral pathogen concentration:** Adenovirus: 2901 genomes/liter in the 4 samples with detectable adenovirus.	12 water samples taken (one from each sampling location) **Measures of association** Pearson linear correlation used. The correlation between "coliphages" and adenovirus was not significant (though r=.32), The presence of human adenovirus was not correlated with the concentration of coliphage. The Tijuana River had the highest concentration of coliphage but a relatively low concentration of adenovirus. However, a correlation between the abundance of human adenovirus and F-specific coliphage was significant, with a correlation coefficient for samples taken from the mouths of the Los Angeles, San Gabriel, Santa Ana, and Tijuana rivers was 0.99.	The presence of adenovirus was not associated with the exceedance of daily limits of bacterial indicators (enterococci, total coliforms, fecal coliforms).

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogen measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Hot, 2003 **Setting** Northern France Four rivers were sampled monthly or semimonthly **Fecal pollutant sources** Rivers impacted by wastewater discharge, but samples were collected upstream of discharge points.	**Coliphage type** Somatic **Measurement method** Somatic coliphages measured using a single-agar-layer method. **Detection** 68 of 68 samples contained measurable somatic coliphage **Concentrations** Mean concentration of somatic coliphages (PFU1^-1) in: River A: 1.9×10^4 River B: 2.2×10^4 River C: 8.5×10^3 River D: 3.3×10^3	**Viral pathogens, measurement methods, and results** Enterovirus measured by culture and endpoint PCR (not ICC n-PCR) Hepatitis A virus, astrovirus, rotavirus, Norwalk I and Norwalk II viruses analyzed by endpoint PCR followed by Southern blot hybridization. **Detection** Culturable enterovirus found in 2 of 68 samples. Other viruses found in 4 of 68 samples. **Concentrations** Enterovirus concentration (most probable number of cytopathogenic units or $MPNCU^{-1}$) in: River A: 33 River B: 6 River C: <1 River D: <1	**Number of observations** 68 water samples were analyzed. **Associations between coliphages and pathogens** Using Student's test, no significant difference was found between somatic coliphage concentration in samples that were positive vs. negative for culturable enteroviruses (P = 0.65) or for enterovirus genomes (P = 0.94 No association between somatic coliphage concentrations and "other enteric viruses" presence.	No fecal indicator bacteria were measured in this study.

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Jiang, 2004 **Setting** Southern California urban rivers and creeks. **Fecal pollutant sources** None of the sites were thought to be impacted by agricultural run-off. At least one river (the San Gabriel River) received tertiary-treated sewage effluent.	A two agar layer system was used. Somatic coliphages were grown on *Escherichia coli* ATTCC 15597 and F+ coliphages were grown on *E. coli* HS(pFamp)R as a specific host for F-specific coliphage. **Coliphage detection** **Coliphage Concentration** Geometric Mean: 119 PFU/100mL Average: 929 PFU/100mL F-specific coliphages: Geometric Mean: 64 PFU/ 100mL Average: 152 PFU/100mL	**Pathogens and measurement methods** Adenovirus, enterovirus, and hepatitis A were identified by endpoint PCR. In the case of adenovirus, nested PCR was performed (without viral culture) Viral pathogen detection: In 21 samples, adenovirus, enterovirus, and hepatitis A virus were detected in 11, 13, and 16 samples, respectively.	21 samples were taken. The relationship between coliphages and enteric pathogens was not explored in the paper. Using data available in Table 3, logistic regression analysis of the presence of each pathogen was conducted. Somatic coliphage was lognormally distributed, and the log10-transformed somatic coliphage values did not approach statistical significance as predictors of either adenovirus, enterovirus, or hepatitis A presence. (Indicator concentrations listed as "less than" were converted to half of the less than (presumably limit of quantitation)	The relationship between fecal indicator bacteria and enteric pathogens was not explored in the paper. Using data available in Table 3, logistic regression analysis of the presence of each pathogen was conducted. Enterococci and fecal coliforms were lognormally distributed, and the log10-transformed concentrations of these indicator bacteria did not approach statistical significance as predictors of either adenovirus, enterovirus, or hepatitis A presence. (Indicator concentrations listed as "less than" were converted to half of the less than (presumably limit of quantitation)

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Skraber, 2004 **Setting** Five sampling locations on the Moselle River in France. **Pollutant sources** The sampling points were varying distances from towns, which were sources of human fecal contamination. Animal fecal sources may have been present as well near some sampling points as one site was noted to be far from such sources.	**Type and method** Somatic coliphage counts were performed according to the standard methods of the International Organization for Standardization. **Somatic coliphage detection:** **Somatic coliphage concentrations** Apparently somatic coliphage was detected in all samples and concentrations were associated with water temperature: Below 15.7 degrees Celsius: 3.29+/-0.59 log PFU/100 mL Above 15.7 degrees Celsius: 2.73+/-0.59	**Viral pathogens and methods** 90 of 170 samples analyzed for infectious enterovirus by cell culture and integrated cell culture endpoint PCR. Norovirus genogroup II were detected using endpoint PCR. Following PCR amplification, viral cDNA was identified by DNA enzyme immunoassays. **Viral pathogen detection:** No cytopathic effect of enterovirus in any of 90 samples cultured. Pathogenic virus nucleic acids were present by the DNA enzyme immunoassay in 38% of samples (enterovirus) and 27% (norovirus)	Figure 3b demonstrates a clear association between ordinal categories of coliphage concentration and percent detection of pathogenic virus genomes, though statistical testing of the association was not performed.	Figure 3a demonstrates a clear association between coliform concentration and percent detection of pathogenic virus genomes, though results of statistical testing of the association were not reported.

Study Setting Fecal pollutant sources	Coliphage measurement method and concentrations	Viral pathogen measurement method Results of pathogen testing	Number of observations Measures of association between coliphages and pathogens	Measures of association between indicator bacteria and pathogens
Ballester, 2005 **Setting** Massachusetts Bay **Fecal pollutant sources** Sampling sites chosen based on proximity to an outfall pipe diffuser head from the Deer Island Sewage Treatment Plant, which releases treated wastewater into the bay. Samples collected every 2 months throughout the 7 year period.	**Coliphage analysis** 1998-1999; EPA Method 1602 (Single Agar Layer Procedure) during From 2000-220 EPA Method 1601 (Two-step Enrichment Procedure). **Concentrations** Coliphage detection increased substantially with the change from single agar layer to two-step enrichment. F+: from 8 to 58% Somatic: from 9.8 to 55%.	**Viral pathogens analyzed** Enterovirus, Adenovirus, Astrovirus, and Rotavirus **Analysis method** 1998-1999: Total culturable virus most probable number (TCV-MPN). 2000-2002: Integrated cell culture-nested PCR (ICC-nPCR). Some of the 1998-1999 samples were re-analyzed using ICC-nPCR. **Results of viral pathogen testing** Concentrations were not given. Instead, detection percentages were utilized.	**Number of observations** Number of water samples not explicitly given, although samples were taken from five sites, bimonthly, for seven years. **Measures of association** Pearson linear correlation to analyze "relationships between organism presence, proximity to the outfall and seasonal variation." Somatic coliphages were correlated (no p-value or confidence level indicated) with enteric viruses (r= 0.573), adenovirus (r= 0.672). Somatic coliphages were not significantly correlated with astrovirus, rotavirus, enterovirus. F+ coliphages were significantly correlated with: Enteric viruses in general (r= 0.682); Adenovirus (r= 0.651); Rotavirus (r= 0.692); Enterovirus (r= 0.608) ; Astrovirus (r= 0.122) (not significant). In 2000–2002 adenovirus was mostly prevalent directly east of the diffuser, rotavirus directly to the west of the diffuser, astrovirus at the shore southwest of the diffuser, and enterovirus at the farthest site in the mouth of the bay.	**Indicator bacteria concentrations** "Indicator bacteria remained below statistically significant counts (<30 cfu per plate)." **Correlation between indicator bacteria and enteric viruses** There was no significant correlation between enteric viruses and indicator bacteria (r= 0).

Study **Setting** **Fecal pollutant sources**	**Coliphage types, measurement method and concentrations**	**Viral pathogens, measurement method and results**	**Number of observations** **Measures of association between coliphages and pathogens**	**Measures of association between fecal indicator bacteria and pathogens**
<u>Choi, 2005</u> **Setting** Southern California Samples taken from two urban rivers, the San Gabriel and Los Angeles rivers. Tertiary-treated sewage is released into the San Gabriel River.	*Escherichia coli* ATCC 15597 strain was used as the host for general coliphages (both somatic and F+coliphage), while *E. coli* Famp was the specific host used for F-specific coliphages. Coliphage samples were mixed with bacteria in soft agar and poured over an LB agar bottom plate. **Coliphage concentration** range: 1 -10^3 PFU/ 100mL F-specific coliphage concentration: 1.0E+00-1.0E+03 PFU/ 100mL	**Viral pathogen** Human adenovirus measured by qPCR. Viral culture using 2 cell lines Enterovirus by endpoint PCR **Viral pathogen detection** Adenovirus by qPCR, detected in 16% of samples. No samples were positive for culturable adenovirus. Enterovirus RNA was detected in 7% of samples. Adenovirus concentration range: 10^2-10^4 genomes/ liter of water	114 water samples were taken. No statistically significant correlations between human adenoviruses and coliphages were identified. .	Fecal coliforms, total coliforms, and enterococci measured by culture. Several correlation coefficients between pathogens and indicator bacteria were presented, but apparently these were not statistically significant, as the text notes that no statistically significant correlations were identified between human adenoviruses and fecal indicator bacteria.

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
<u>Moce´-Llivina, 2005</u> **Setting** Two bathing beaches in Barcelona, Spain. **Pollutant sources** The beaches are impacted by municipal wastewater effluents via underwater outfalls and secondary treatment effluents from small towns via rivers that flow into the sea near the beaches.	Somatic and F+ coliphages measured using a double-layer technique, using standards from the International Organization of Standardization. F+ RNA coliphages genotypes using oligonucleotide hybridization. **Coliphage detection** Somatic coliphage in 20/20 samples. F+RNA coliphage in 3/20 samples. **Coliphage concentration** Somatic coliphages (average of 20): 743.75 PFU/100mL Coliphage type: 18% genogroup I , 82% genogroup II (Bacteriophages that infect *Bacteroides thetaiotamicron* detected in 14/20 samples)	Enterovirus was cultured using three methods and then detected by endpoint PCR. Enterovirus was also detected in seawater samples by endpoint PCR. In samples tested by the 3 viral culture methods, VIRADEN resulted in the most frequent detection (8 of 11 samples). Enterovirus present in 4 of 20 samples by endpoint PCR; in 10 of 18 samples by culture. Enterovirus concentrations in culture were generally 1-4 PFU/ 10 Liters, though two samples had concentration about 50 times higher than that.	20 water samples were utilized. A ROC curve using the criteria “numbers of enteroviruses in 10 liters of seawater” and indicators. Somatic coliphage produced a curve with an area of 0.63, indicating predictive value, though it’s not clear what the dichotomous outcome enterovirus variable was (presumably presence vs. absence).	A ROC curve using the criteria “numbers of enteroviruses in 10 liters of seawater” and indicators. Enterococci produced a curve with an area of 0.7, indicating predictive value, though it’s not clear what the dichotomous outcome enterovirus variable was.

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Jiang 2007 **Setting** 15 locations in Newport Bay, California. Some were tributaries that flow into the Bay and historically had high fecal indicator bacteria concentrations. Other sites included bathing beaches. **Fecal pollutant sources** The Bay receives runoff from a large, mixed, urban and agricultural watershed.	**Coliphage analysis method** F+ coliphage, by two-step enrichment (EPA method 1601). **Coliphage presence** **Coliphage concentration** F+ coliphage was reported as presence/absence due to the upper limit of quantification.	**Viral pathogen analysis** Enterovirus, detected by endpoint PCR. Adenovirus, detected by nested PCR (endpoint). Culture of viral pathogens was not performed. **Viral pathogen presence** Perhaps due to recovery limitations, human adenoviruses was detected in 4.3% and enteroviruses in, 4.8% of all samples.	**Observations** 206 samples analyzed The partial correlation analysis, controlling for temperature, salinity, and sampling data, showed that the seasonal detection of human adenovirus and enterovirus was negatively correlated to coliphage. However, these correlations were statistically insignificant (P >0.05). Similarly, no statistical relationship was apparent within sampling sites between human viruses and coliphage.	The partial correlation analysis, controlling for temperature, salinity, and sampling data, showed that the seasonal detection of human adenovirus and enterovirus was negatively correlated to that of FIB. However, these correlations were statistically insignificant (P > 0.05). Similarly, no statistical relationship was apparent within sampling sites between human viruses and fecal indicator bacteria.

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Espinosa, 2009 **Setting:** Southern Mexico City Surface water in urban settlement, taken from 10 locations along a network of canals used for irrigation as well as 10 drinking water wells. No wastewater mentioned, but water used for irrigation may have domestic animal and human contamination.	**Coliphage measurement** Double layer agar method. Not stated whether somatic, F+ or both. **Coliphage detection** Present in 40 of 80 samples. **Coliphage concentration** Not reported	**Pathogens and measurement method** Enterovirus, rotavirus, astrovirus measured by endpoint PCR. **Viral pathogen detection** Rotavirus and enterovirus present in approximately 30% and 60% of cold, dry-season canal samples, respectively; about 10% of warm, wet season samples.	80 water samples analyzed. Coliphages found to be associated with enterovirus (p-value: 0.0182), but not with rotavirus or astrovirus (p-values: 0.150 and 0.459).	Although *E. coli*, total coliforms, enterococci, were measured, associations between the bacteria and viral pathogens were not reported.

Study **Setting** **Fecal pollutant sources**	**Coliphage measurement method and concentrations**	**Viral pathogens and measurement methods; Results of pathogen testing**	**Number of observations** **Measures of association between coliphages and pathogens**	**Measures of association between fecal indicator bacteria and pathogens**
<u>Jurzik, 2010.</u> **Setting** Germany, five sites along the river Ruhr. **Pollutant sources** Five sampling sites ranged from 1.5 to 10 km downstream of the nearest sewage plant.	**Method** Somatic coliphages were quantified using a double layer plaque assay using a method of the International Organization for Standardizatio n. **Concentrations** Somatic coliphage concentrations were found to have a range of 3.0-8.1 x 10^4 PFU/L.	**Method** Quantitative PCR (not ICC n-PCR) **Concentrations** Detection frequency: enterovirus (17.8%), norovirus genotype II (25.7%), rotavirus (63.5%) human polyomavirus (68.6%), human adenovirus (96.3%) Concentration Range (in genome equivalents per liter or gen.equ./L) for: Adenovirus: 5.7 x 10^1 to 7.3 x 10^5 Enterovirus: 1.0 x 10^2 to 1.1 x 10^6 Norovirus GII: 3.1 x 10^1 to 6.4 x 10^4 Rotavirus: 1.6 x 10^1 to 3.8 x 10^5 Polyomavirus: 3.7 x 10^1 to 5.2 x 10^5	**Number of samples** Number analyzed for individual viruses ranged from 174 (enterovirus) to 190 (human adenovirus) Pearson correlation coefficients were reported within strata of water temperature: Somatic Coliphages were found to have the following r values with: Adenovirus: -Below 10 degrees C: 0.27 -Equal or Above 10 degrees C: -0.07 -At all temperatures: 0.12 Polyomavirus: -Below 10 degrees C: -0.07 -Equal or Above 10 degrees C: 0.41* -At all temperatures: -0.03 Rotavirus: -Below 10 degrees C: -0.04 -Equal or Above 10 degrees C: -0.07 -At all temperatures: -0.05 *P<0.05 (none of the other correlation coefficients listed above were statistically significant at p<0.05)	Using a Pearson correlation, r values were found for the relationships between: *E.coli* and polyomavirus: --Equal or Above 10 degrees C: 0.49* *E. coli* and Rotavirus: --Below 10 degrees C: 0.46* --At all temperatures: 0.31* Total coliforms and polyomavirus: --Equal or Above 10 degrees C: 0.67* Total coliforms and rotavirus: --Below 10 degrees C: 0.46* --At all temperatures: 0.29* Enterococci and polyomavirus: --Equal or Above 10 degrees C: 0.41* *P<0.05 Correlations between the fecal indicator bacteria and human adenovirus were not significant. No other correlations between the FIB and viral pathogens were observed at a p=0.05 level of significance.

Study Setting Fecal pollutant sources	Coliphage measurement method and concentrations	Viral pathogens and measurement methods; Results of pathogen testing	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
<u>Lodder, 2010</u> **Setting** The Netherlands, 10 locations were sampled, either intake areas of drinking water companies, or upstream of source water intake areas. **Pollutant sources** Several river locations (Maas and Drentsche Aa catchments) had wastewater treatment plants located upstream of sampling locations.	**Method** Concentrations were determined via a monolayer plaque assay. **Concentrations** Somatic and F+ coliphage detected in 100% and 97% of samples, respectively. Range of mean somatic coliphages in each location: 105 to 1.7 x 10^4 PFU/liter Range of mean F-specific phages in each location: 2.0 to 4.3 x 10^3 PFU/liter	**Viral pathogens and measurement methods** Enterovirus, reovirus: BGM cell culture (but not ICC nPCR) Norovirus, rotavirus: RT-PCR **Results** Enterovirus, reovirus, norovirus, and rotavirus detected in 75%, 83%, 45% and 48% of samples, respectively. Range of mean concentrations: Enterovirus: 0.0052 to 2.4 PFU/liter Reovirus: 0.013 to 1.3 PFU/liter Norovirus: 0 to 26 PCR-detectable units (PDU)/liter Rotavirus: 0.88 to 375 PDU/liter Problem with qPCR method to quantify viruses: in 49% of samples, could not differentiate non-detect from interference.	**Number of observations** 75 water samples were taken (total) form 10 locations over a 4-year period. **Measures of association** A correlation between the presence enterovirus and coliphages was highly significant (< 0.0005) but the correlation coefficient was not reported. None of the other viral pathogens were correlated with coliphages. In the two samples that tested negative for F+ coliphage, enterovirus was present, and in one of those two samples, norovirus and rotavirus were also present.	Fecal indicator bacteria were not evaluated

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
<u>Haramoto, 2011</u> **Setting** Kathmandu Valley, Nepal Samples were taken from nine shallow groundwater wells, as well as one river. **Fecal pollutant sources** These well sites are located near toilets, and are suspected by the authors of failing to meet WHO guidelines for microbiological contamination. The river contains human fecal pollution.	**Coliphage types** F+ RNA coliphages and genogroups **Measurement method** qPCR **Results** Concentration (gene copies/volume of water) not quantified, just CT values detection of coliphages via qPCR was performed, and the cycle threshold was recorded. **Concentrations** F+ coliphage detected in 3 well and the 1 river samples. Results CT range for: F-Specific Coliphage: Genogroup I: 39.2 (one location) Genogroup II: 31.9-38.9 Genogroup III: 38.8 (one location)	**Viral pathogens** Adenovirus, norovirus **Measurement method** RT-qPCR **Other pathogens** Samples were also analyzed for Giardia and Cryptosporidium. **Results** All pathogens were detected in the river sample. Viral or protozoan pathogens were detected in 1-3 wells (depending on the particular pathogen of interest). **Concentrations** (gene copies/volume of water) not quantified, just CT values CT range: Adenovirus: 34.3+/-0.4 – 41.5 Norovirus: Genogroup I: 36.7+/-0.1 – 36.8+/-0.1 Genogroup II: 34.0+/-0.6 – 37.5+/-0.6	**Number of observations** 10 water samples were analyzed. Statistical testing of associations between F+ RNA coliphages and the enteric viruses was not reported. Of the two samples that tested for positive adenovirus, one tested negative for coliphage and one tested positive for coliphage. Of the two well samples that tested positive for norovirus, both were positive for coliphage. Of the three well samples that tested positive for coliphage, one was negative for the viral pathogens.	Total coliforms detected in all samples; *E. coli* detected in 7 of 10 samples. The (33%) of the nine wells tested had no detectable *E. coli* and none of these samples tested positive for the viral or protozoan pathogens. Five of the six *E. coli*-positive samples were positive for the pathogens ($p < 0.05$).

Study Setting Fecal pollutant sources	Coliphage measurement method and concentrations	Viral pathogens and measurement methods; Results of pathogen testing	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
<u>Love, 2014</u> **Location** Doheny Beach and Avalon Beach, Southern California **Fecal pollutant sources** Beaches were thought to be potentially impacted from non-point source pollution. A previous study suggested that a leaky sewage system contaminates groundwater and then the sewage contamination can then enter Avalon Bay during outgoing tides.	**Coliphage analysis and concentrations** A modified version of US EPA Method 1601 (two step enrichment) was used for F+ and somatic coliphage detection. Coliphage presence vs. absence was converted to most probable number (MPN). **F+ coliphage concentration:** At Doheny Beach: Median 0.3 (range <0.09 to 140) MPN/ 100mL At Avalon Beach: Median 4.9 (range <0.01 to 37) MPN/ 100mL **Somatic coliphage concentration:** At Doheny Beach: Median 4.9: (range <1 to 150,000) MPN/ 100mL At Avalon Beach: Median (range <1 to >370) 3.1 MPN/ 100mL	**Viral pathogens and measurement methods** PCR (adenovirus) and RT-PCR (Norovirus) but not ICC-nPCR **Results of pathogen testing** Adenovirus and norovirus were detected in about 22% of samples at Doheny beach and in 9.3% and 0.7% of samples, respectively, at Avalon beach.	**Number of observations** 324 water samples were taken in total (multiple per sampling day during intensive sampling). A Generalized Estimating Equation model was used to determine associations between coliphages and enteric viruses. At Doheny Beach, the probability of detecting adenovirus was greater in the absence of F+ coliphages (inverse association; p=0.002, OR=0.24), and had no significant association with somatic coliphages. Norovirus was not significantly associated with either type of coliphage. At Avalon Beach, detecting a direct association between adenovirus and F+ coliphages was suggested (OR=1.98) though this was of marginal statistical significance (p=0.1). No mention is made of somatic coliphages at this beach.	At Doheny Beach, the probability of detecting adenovirus was greater in the absence of enterococci (p=0.001, OR=0.24), and in the presence of fecal coliforms (p=0.02, OR=1.004). Norovirus was not significantly associated with any fecal indicator bacteria. At Avalon Beach, the probability of detecting adenovirus was associated with higher fecal coliform concentrations (p=0.01, OR=1.99) as well as total coliform concentrations (p=0.002, OR=1.44). Norovirus was not included in the modeling for this beach.

Study Setting Fecal pollutant sources	**Coliphage types, measurement method and concentrations**	**Viral pathogens, measurement method and results**	**Number of observations Measures of association between coliphages and pathogens**	**Measures of association between fecal indicator bacteria and pathogens**
Liang, 2015. **Setting** Singapore Surface water in urban storm water catchments, as well as from rivers and canals. No explicit mention of contamination sources.	**Coliphage types** Somatic, F+ **Method** US EPA Method 1601 (single-agar-layer). **Concentrations** Geometric means Somatic coliphage: 52 PFU/100mL F+ coliphage: 27 PFU/100mL Other fecal indicators analyzed *B. thetaiotaomicron, M. smithii,* and human polyomavirus, all by qPCR; *E. coli* and enterococci by both culture and qPCR.	**Viral pathogen measurement method** qPCR, not by ICC n-PCR **Other enteric pathogens analyzed:** *P. aeruginosa* and *Salmonella spp.* by culture. **Viral pathogen presence** Viral nucleic acids detected in 20-48% of samples (depending on the virus) **Results (geometric mean gene copies/L)** Rotavirus: 11 Astrovirus: 57 Norovirus GI: 7 Norovirus GII: 104 Adenovirus: 13	148 water samples taken Spearman's ranks correlation between F+ or somatic coliphage and viral pathogens: None significant at p<0.05 level. Coliphages associated with *Salmonella* and *P. aeruginosa.*	Spearman's rho correlation coefficients (significant at p<0.05, two-tailed t-test): *E.coli* (qPCR) and norovirus GII: 0.453 *E. coli* (qPCR) and adenovirus: 0.372 Enterococci (qPCR) and norovirus GII: 0.487 Enterococci (qPCR) and adenovirus: 0.637 *B. thetaiotaomicron* and norovirus GII: 0.421 *M. smithii* and norovirus GI: 0.397 *M. smithii* and norovirus GII: 0.411 Human polyomavirus and norovirus GI: 0.440 Multiple linear regression models of norovirus GII concentrations: *E.coli*: r^2=0.153; Model Significance=0.02 Enterococci: r^2=0.442; Model Significance=0.000 *M. smithii*: r^2=0.762; Model Significance=0.000;

Study Setting Fecal pollutant sources	Coliphage types, measurement method and concentrations	Viral pathogens, measurement method and results	Number of observations Measures of association between coliphages and pathogens	Measures of association between fecal indicator bacteria and pathogens
Updyke, 2015. **Setting** 18 locations in Hawaii, sampled six times to evaluate seasonal effects on microbe concentration. **Pollutant sources** Some sampling locations were near sewage treatment plants.	**Coliphage types** F+ RNA **Method** PCR Other fecal indicators analyzed (by PCR) *E. coli*	**Viral pathogen measurement method** Enterovirus, norovirus genogroup I, norovirus genogroup II By PCR. Samples that tested positive for enterovirus were analyzed by culture. **Viral pathogen presence** 31 samples were positive for enterovirus on PCR. All of these showed no infectivity on culture.	108 water samples analyzed (18 sites). Samples from six of the sites (36 samples) were used in analyses of indicators and enteric viruses. Coliphage-enteric pathogen associations were not reported. Of six sites that were each tested twice, two locations tested positive (once each) for F+ coliphage. Of the 10 days/sties of negative coliphage results, 7 were positive for enteric viruses (Table 3)	No significant associations.

Coliphages as indicators of health risk

The EPA's literature review of coliphages as indicators of fecal contamination summarized the results of eight epidemiologic studies. One of those studies, "Griffith et al. (personal communication, 2015)," remains unpublished and the methods and details of the findings could not be reviewed. The other seven, along with a study that was published in late 2015 (Dorevitch et al., 2015) are summarized below. Basic aspects of studies and findings are provided in Table 2 and details of individual studies follow. Note that Dorevitch is the author of this review and is not the ideal person to critique that study.

Level One: Overall summary of coliphage and health risk

As presented in Table 2, one epidemiologic study found that coliphages but not FIB predict illness (Colford et al.); one study found that FIB but not coliphages predict illness (Van Asperen); three studies found that both FIB and coliphage predict illness (Lee et al., Wiedenmann et al., Wade et al.); one study found that neither FIB nor coliphage predict illness (Abdelzaher et al.); one study found that coliphage predicts illness in some settings/conditions while FIB did not (Dorevitch et al.); and one study did not summarize data analysis in a way that would provide an answer to this question (von Schirnding et al.). It should be noted that the epidemiologic studies varied substantially in study design, recreational activity, exposure definitions, water quality, laboratory methods, and data analysis methods. To summarize, there is little consistency in the epidemiologic literature regarding whether coliphage concentrations predict illness following water recreation. This is in contrast to the general consistency among the epidemiologic studies that at beaches impacted by wastewater, fecal indicator bacteria do predict illness.

Study	Impacted by wastewater treatment plant?	Size*	Coliphage was an indicator of health risk?	FIB was an indicator of health risk?
Von Schirnding, et al., 1992 [39]	No, but beaches were impacted by local sources of untreated human fecal pollution	Medium	Not determined because concentrations of coliphage were "non-significant"	Not reported. Rate of illness at the beach with higher FIB concentrations tended to be higher than at the beach with lower FIB, but this was not statistically significant.
Lee et al., 1997 [40]	The concrete whitewater slalom course was fed in part by wastewater	Medium	F+ coliphage: Yes	Yes, but not independently of coliphage
van Asperen et al, 1998 [34]	Yes, by treated domestic sewage	Medium	F+ coliphage: No	Yes
Wiedenmann et al., 2006 [41]	Yes, some sites were impacted by sewage discharge and combined sewer overflows	Medium	Somatic coliphage: Yes	Yes
Colford et al., 2007 [42]	Not impacted by point sources	Large	F+ coliphage: Yes Somatic coliphage: No	No
Wade et al, 2010 [6]	Beaches were thought to be impacted by wastewater discharge	Large	On days of higher coliphage concentrations, illness rates among swimmers were higher than rates among non-swimmers. Risk among swimmers was not associated with coliphage measures at a $p<0.05$ of statistical significance.	Yes
Abdelzaher, et al 2011 [43]	No	Medium	Somatic coliphage: No	No
Dorevitch 2015 [36]	Effluent-dominated	Large	Somatic, F+: No	No
	Not effluent-dominated	Medium	During dry weather: Borderline significance	No
	Not effluent-dominated	Large	During wet weather: No	No

Table 2: Summary of epidemiologic studies and indicators as predictors of illness

***Study size: Small <250 water-exposed study participants; Medium: 250-999 water-exposed study participants; Large: ≥1,000 water-exposed study participants**

Level Two: Coliphage and health risk literature details

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
Von Schirnding, 1992 [39] **Setting and fecal pollutant sources** Two beaches in Cape Town, South Africa. One was impacted by septic systems, storm runoff, and fecal pollution from a river. **Design** Cohort enrolled at two beaches of differing water quality **Participants** "Swimmers" exposure above the waist to beach water N=478 "Non-swimmers" Exposure to beach water below waist only, OR no water contact. N=254	**Sampling**: three locations/beach, before and during peak use **Fecal indicator bacteria concentrations** Fecal coliforms: Median 76.5/100mL and 8.0/100mL at the two beaches Enterococci: Median 51.5/100mL , 2.0/100mL at the two beaches **Coliphage measurement** Plaque assay using *E. coli* strain C as host Coliphage and *S. aureus*: "insignificant densities were detected"	**Rate of GI illness** (defined by phone interview 3-4 days after index exposure) GI symptoms were reported in about 4% of swimmers at the more polluted beach, and 2% of non-swimmers at the less-polluted beach, and among swimmers and non-swimmers at the less-polluted beach. Differences not statistically significant. At the more polluted beached, FIB concentrations were higher than at the less polluted beach Fecal coliforms median 75.6 vs. 8.0 CFU/100mL Enterococci 51.5 vs 2.0 CFU/100mL **Fecal indicator bacteria as predictor of GI illness** Not reported	Could not be evaluated as coliphage densities were "insignificant"

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
Lee et al., 1997 [40] **Setting** Whitewater slalom course fed in part by wastewater **Design** Cohort study of canoeists and rafters on 11 dates (1-2 month, March –Nov), symptom follow-up 1 week later N=473 water users (no unexposed group) who completed 1-week follow-up questionnaire (out of 755=63%)	**Water sampling** Hourly **F+ coliphage analysis** Grown on *S. typhimurium* Range of daily means 1-99, median 26 PFU/100mL **Enterococci** Range of daily means 7-3,963; median 102 CFU/100mL **Enterovirus** Exceeded 4PFU/10L on only one occasion	Rates of illness: Diarrhea reported by 2-15% of participants on 8 different days of the study; median=7.5%. Densities of either *E. coli* or *S. faecalis* were significant predictors of GI illness in models that did not include F+ coliphage. However, after taking into consideration F+ coliphage densities, *E. coli* and *S. faecalis* were no longer significant predictors.	**Relative risk by F+ coliphage** PFU/volume (reported as per 10mL but probably should have been reported as per 100mL) 1-3: 1.0 26-32: 2.6 69-308: 2.8 **Association between F+ coliphage and enterovirus** On 9 dates both were measured. No meaningful correlation, with R^2=0.04 (Log10 transformed, R^2=0.008)

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
van Asperen et al., 1998 [44] **Setting** Netherlands inland waters **Design** Cohort with comparison group **Participants** Swimmers (1 or 1.5 km distance) in Olympic distance triathlon. N=827 with completed f/u questionnaires (62.3% response rate) *Non-water recreators* Participants in run-bike-run events. N=773 with completed f/u questionnaires (62.0% response rate) **Fecal pollutant sources**	**Sampling** Done at multiple locations during events. **Coliphage analysis** F+ RNA coliphage analysis using ISO DIS 10705-1. **Concentrations** F+ RNA coliphage concentration Geometric mean=0.7/L Range <0.001, 13.6/L (n=31 samples) E. coli: Geometric mean =204/10mL Fecal strep: Geometric mean =16	**Rates of illness** Based on information recorded in a symptom diary for 6 days after event. Non-swimmers (run-bike-run participants) Illness (GI-UK definition) rate=1.7/100 Swimmers (triathletes) Illness (GI-UK definition) rate=3.6/100 **FIB concentrations and illness** Increased illness incidence with increasing microbe concentrations above a threshold. For thermotolerant coliforms and *E. coli,* threshold estimated as GMs of 220/100 mL and 355/100 mL, respectively. Association between illness and these indicators as dichotomous variables (above vs. below threshold) statistically significant. As continuous variables on a $\log_{10}$ scale, correlation significant, but R^2 or parameter estimate not presented. No association between GI illness attack rate (per study date) and fecal strep or enterovirus.	Triathletes more likely to develop illness. Adjusted odds ratios varied by definition of illness, GI-UK, 1.6 (0.8 – 3.2) No association between GI illness attack rate (per study date) and coliphage concentration.

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
<u>Wiedenmann, 2006 [41]</u> **Setting:** Beaches at five freshwater sites in Germany, 4 lakes and one river; multiple potential fecal pollution sources **Design:** Randomized controlled exposure trial **Exposure** 10 minutes in the water exposure; minimum of 3 head immersions. Could also swim, play in the water Unexposed group: stayed in grass/sand area, no water contact **Number of study participants** Final cohort analyzed=1,759; not stated how many were water-exposed	**Water sampling** Every 20 minutes during exposure trails **Coliphage analysis** Double agar layer method **Mean 'microorganisms' reported per 100mL** (approximately 420 samples, except Aeromonads, which was tested in 385 samples) *E. coli* : 136/100mL Intestinal enterococci: 37/100mL *C. perfringens*: 18/100mL Aeromonads: 8,200/100mL *P. aeruginosa*: 10/100mL Somatic coliphage: 20 /100mL Researchers defined an optimized threshold for each microbe that differentiates elevated risk from background risk, and referred to this as the no observed adverse effect level (NOAEL)	**Rates of gastrointestinal illness** Illness rate 1 week after the exposure was described using three definitions of illness. This summary uses the one most similar to the definition in recent US epidemiologic studies (GE_UK) Unexposed: 1.4/100 bathers Exposed: 3.3/100 bathers Proposed NOAEL, microorganisms per 100mL: (using the authors' "definition 1" of exposure, which does not take into account the number of times an individual immersed their head) *E. coli:* 180/100mL Intestinal enterococci: 24/100mL *C. perfringens*: 13/100mL Attributable risk % of GI illness (GE_UK) above NOAEL vs non-swimmer: *E. coli* : 3.6 Intestinal enterococci: 3.1 *C. perfringens*: 3.3 Good evidence of a dose-response relationship between ordinal measures of *E. coli*, intestinal enterococci, *C. perfringens* and a somewhat different definition of gastroenteritis (does not include stool frequency) No associations between illness and Aeromonads, *P. aeruginosa.*	Proposed NOAEL, microorganisms per 100mL: (using the authors' "definition 1" of exposure) Somatic coliphage: 150/100mL (authors note that this estimate may not be accurate because of non-normal distribution of coliphage measurements) Attributable risk % of GI illness, above NOAEL vs non-swimmer: Somatic coliphage: 5.1

Author, Year, Setting, study design	Participants: Water, non-water Illness rate	Water sampling and microbe concentrations	Coliphage test type	Findings	Findings: other WQ measures and health
Colford 2007 [42] Six marine beaches thought to have little point-source fecal pollution, Mission Bay, CA Cohort study, 14-day telephone follow-up	4,234 with complete telephone data with somatic coliphage data (Appendix E) Illness rate among swimmers: HCGI-1 Illness rate among non-swimmers: 2.3% HCGI-1 Illness rate among swimmers: 2.9%	Enterococcus, culture GM=29 MPN/100mL Enterococci qPCR GM=65 CCE/100mL F+ coliphage: Detected in 16/141 samples(GM 0.2/100mL max: 0.78/100mL) Somatic coliphage: Detected in 96/141 samples GM 0.6 /100mL Max: 36.6 /100mL) Adenovirus: detected in 1/151 samples Norovirus: detected in 0/151 samples	EPA Method 1601	Somatic coliphage: no association Based on data in Appendix E, odds ratio for association between detectable F+ coliphage and diarrhea OR (95% CI = 1.04 (0.50 to 2.15), p=0.91 Multivariate logistic models of illness that considered F+ coliphage as a continuous measure: Diarrhea 1.1 (0.97–1.4) HCGI-1 1.3 (1.1–1.5) HCGI-2 1.4 (1.1–1.8) Nausea 1.3 (1.2–1.6) Cramps 1.0 (0.83–1.3) Vomiting 1.2 (0.96–1.5)	Illness not associated with measures of enterococci (culture or qPCR), fecal coliforms, total coliforms.

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
<u>Wade et al., 2010 [6]</u> **Setting** NEEAR study Marine beaches affected by POTWs 2005: Mississippi, 2007: Alabama, Rhode Island **Design** Cohort study. Summary limited to the two beaches at which in 2007 coliphage testing was conducted Immersion to waist or higher ("swimming exposure"): 1,903 No immersion of body: 3,802 Based on data in Table 5: Non-swimmer=1,776 Swimmer=1,335	**Sampling** 3 transects and 2 depths/beach , 3 times/day **Bacteria concentrations per 100mL Enterococci measured by culture ; qPCR** Alabama: GM=21 CFU ; 260 $CCE_{\Delta\Delta}$ Rhode Island: GM= 3.6 CFU; 160 $CCE_{\Delta\Delta}$ **Coliphage testing methods** 1) CLAT presence/absence for F+ RNA, F+ DNA coliphages 2) Method 1601 spot test (F+) **Coliphage concentration** *Fairhope Beach:* Coliphage detectable in 56% of samples by 24-hour SPOT assay 4%, 14% detectable for F+ RNA, F+ DNA by CLAT *Goddard Beach:* Coliphage detectable in 65% of samples by 24-hour SPOT 8%, 9% detectable for F+ RNA, F+ DNA by CLAT	**Rate of GI illness** Non-swimmers: 5.6 cases per 100 Swimmers: when enterococci (culture) was < 2.32 CFU/100mL, 7.39 cases per 100 swimmers: when enterococci was >22.9 CFU/100mL, rate 11.46 per 100 **Enterococci culture results as predictors of illness** Rates of illness among swimmers statistically equivalent whether enterococci culture above vs below 35CFU/100mL. Rates of GI illness and, diarrhea significantly greater among swimmers when enterococci >35 CFU/100mL compared to rate among non-swimmers. The odds of GI illness is not statistically increased for a log_{10} increase in enterococci culture results. **qPCR results as a continuous variables, as predictors of illness** Log_{10} qPCR results for Enterococcus, Bacteroidales, Bacteroides, and Clostridium were significant predictors of GI illness, though for some of these analyses, the association was dependent on the method of calculating qPCR results.	**Coliphage as an ordinal measure, vs. non-swimmers** F+ coliphage spot assay Concentration: 0.1 – 0.7/100mL: Odds ratio 1.52 (0.98, 2.36) Conc. F+ 0.7 – 2.4/100mL: Odds ratio 1.70 (1.12 – 2.57) **F+ coliphage detection (CLAT) as predictor of illness measure, vs. non-swimmer** Not detected: 1.18 (0.74-1.88) Detected: 1.80 (1.22, 2.66) F+ DNA CLAT not detected: 1.11 (0.64 -.93) F+ DNA CLAT detected: 1.69 (1.16 – 2.47) **F+ coliphage spot assay a s a continuous variable among swimmers** Adjusted odds ratio for illness among swimmers for a 1-log increase in F+ RNA coliphage: 1.15(0.69-1.92) **Findings: CLAT assay results, as a continuous variable among swimmers** Adjusted odds ratio for illness among swimmers for a 1-log increase in F+ RNA coliphage: 1.55 (0.9 – 2.66) Adjusted odds ratio for illness among swimmers for a 1-log increase in F+ DNA coliphage: 1.61 (0.86 – 3.0)

Study, setting, study design, participants	Water sampling, analysis, and measured microbe concentration	Health risk findings : rates of illness, FIB as predictors of illness	Coliphage as a predictor of gastrointestinal illness
Abdelzaher, et al. 2011 [43] **Setting** Subtropical , non-point-source marine beach in Southern FL **Design** Randomized controlled exposure to head immersion **Participants** Bathers: 15 min in water with at least 3 head immersions N=652 Non-bathers: 15 minutes on the beach N=651	**Sampling** Composite of 30-60 bather-collected samples per 3.5 hours, as well as "investigator-collected" composite samples. **Coliphage analysis** Single layer agar method for somatic (referred to as F-coliphages in the paper and supplement) and F+ coliphages **Coliphage concentrations** F+ coliphage not detected in any sample (<0.3 PFU/100mL) Enterococci: <2-109 CFU/100mL	GI illness rate difference between bathers and non-bathers: 2 per 100 Somatic coliphage detected in 3 of the 5 (of 15 total) days with biggest differences in GI illness rates in bather vs. non-bather. No statistically significant associations between coliphages and the difference in illness rates between bathers non-bathers. This may be because the number of observations (study days) was only 15.	No statistically significant associations between any measure of WQ and the bather-non-bather illness rate difference

Author, Year, Setting, study design	Participants: Water, non-water Illness rate	Water sampling and microbe concentrations	Findings	Findings: other WQ measures and health
Dorevitch, 2015 [45] **Setting**: Chicago area surface waters (freshwater) including the heavily polluted Chicago River system; rivers, small lakes, Lake Michigan **Design** Cohort study of incidental contact water recreation;	**Participants** 4,929 water recreators free of baseline GI symptoms with health follow-up and coliphage data **Illness rate** 4.30/100 at effluent-dominated waters 4.25/100 at general use waters	**Water sampling** Every two hours during water recreation. **Coliphage test method** EPA Method 1602 Median concentration (per 100mL) Enterococci: 126.6 CFU Somatic coliphage 31.7 PFU F+ coliphage: 1.7 PFU Giardia cysts: 0.008	Association with GI illness during dry weather at waters not dominated by wastewater effluent only. Somatic coliphage: OR 1.01 (1.00, 1.02) and F+ coliphage: 1.05 (0.96, 1.14) (borderline statistical significance); at those waters during wet weather: No association. At effluent-dominated waters: No association between illness rate and either F+ or somatic coliphage.	No association between other water quality measures and illness.

Information in the above detailed tables above is not entirely in agreement with that contained in Table 4 of the EPA's review. While these are not likely to have substantive impacts of evaluations of coliphages as water quality indicators, for completeness they are summarized below.

Study	EPA review – Table 4	A more complete statement
Wiedenmann 2006 [41]	Column 3: "Significantly increased RR of gastroenteritis for bathing in waters with somatic coliphage levels above the NOAEL (10 PFU per 100 mL) versus nonbathing."	Using the definition that is closest to the NEEAR GI illness definition (three diarrheal stools/24 hours) - UK_GI - the NOAEL is 150PFU/100mL
Colford 2007 [42]	Column 1: Sample size=8,000 Supports coliphages as water quality indicator? (Column 4): Yes, F-specific coliphage	4,234 with complete telephone data with somatic coliphage data (Appendix E) For completeness, Column 4 should read: Yes, F-specific coliphage No, somatic coliphage
Wade 2010 [6]	Sample size =6,350 Column 4: Yes; F-specific coliphage	The number 6,350 was indeed reported in the publication. However, data from relatively few of those participants were included in the coliphage analysis. Number of swimmers included in the analysis of coliphage data: 1,335 (based on information in Table 5 of the publication). Coliphage analyses that used the EPA reference method ("spot") were not associated with GI illness. The comparison group used in the analysis of coliphage data generated by the CLAT method (Column 3 of the Table 4, EPA Coliphage report) was non-swimmers. In EPA analyses of qPCR as predictor of risk among swimmers, swimmers exposed to a range of qPCR measures of water quality were analyzed (with qPCR results on a $\log_{10}$ scale). The use of a similar approach for the CLAT results showed no statistically significant association between GI illness and either log10 F+RNA or F+ DNA coliphages. By contrast, log10 transformed qPCR results were predictive of GI illness among swimmers (with a larger sample size).
Abdelzaher 2011 [43]	Column 4 "Somatic coliphage detection overlaps with highest illness days"	Health data from this study were analyzed very differently than those of the EPA studies. As analyzed, there were only 15 observations (one for each day of the study). Of the five days with the greatest difference in swimmer and non-swimmer illness rates, three had detectable coliphages

		and two did not. It's not clear that this should not be considered support for coliphage (Table 4). Better to note: No support, though study too small to detect weak or moderately strong associations.
Griffith 2015		Consider waiting until the information has been published in the peer-reviewed literature before including it in the review.

Table 3: Areas of incomplete agreement between information in the EPA review Table 4 and the present review of epidemiologic studies of coliphages and water recreation.

Comparison of studies used to develop recreational water quality criteria and in the review of coliphages

In the EPA's epidemiologic study that evaluated coliphages and water recreation health risks, 1,335 water-exposed participants were enrolled for whom coliphage data were available (Table 4) at marine beaches. The table puts this information into the context of the number of water-exposed study participants in EPA's epidemiologic studies conducted in support of prior recreational water criteria development.

	1986 Criteria AWQC [9] (for Enterococci, E. coli by culture)	2012 RWQC [10] Enterococci by qPCR	Coliphage
Number of marine beaches, swimmers, other water recreators	1986 AWQC, Table 1: New York City (3 summers): 9,463 L. Pontchartrain (3 summers): 4,768 Boston Harbor: (1 summer): 2,049 Total: 16,280	Wade 2010, "Immersion" participants (Table 1): Edgewater (2005): 741 Fairhope (2007): 823 Goddard (2007): 1,080 Total 2,644	Wade 2010 [6] Table 5: Fairhope and Goddard beaches (2007 only): 1,335
Number of freshwater beaches, swimmers, other water recreators	Lake Erie (3 summers): 14,784 Keystone Lake (2 summers) 14,182 Total: 28,966	Wade 2008 Limited to swimmers included in the qPCR-health risk analysis (Appendix C) Swimmers: 9,327	None
Marine + freshwater	**45,246**	**11,971**	**1,335**

Table 4: Number of swimmers with health data analyzed in relation to coliphage data in US EPA epidemiologic studies

Viral pathogens as predictors of illness in epidemiologic studies

The third question that this report is meant to answer is, "Is there a relationship between enteric viruses and human health in recreational waters? If so, what is that relationship?" Table 5 summarizes this information. The two studies that were not described previously in this report (Fewtrell, 1992 and Hale, 1999) are summarized in the following section.

Study	Pathogen	Associated with illness?
Fewtrell et al., 1992 [46]	Enterovirus	Yes
Lee et al., 1997 [40]	Enterovirus	Not reported
Van Asperen et al., 1998 [44]	Enterovirus	No
Haile et al., 1999 [47]	Human enteric virus	Borderline significance
Colford et al., 2007 [42]	Adenovirus, norovirus	No

Table 5: Cohort studies of enteric viruses and human health in recreational waters

Fewtrell [46]: White water canoeists were enrolled at two courses: Course A was the same site studied in Lee 1997. At Course A, enterovirus measured by culture was detected in 10/10 sample, with a mean concentration of 198.4 PFU/10L. At Course B, which receives water not impacted by wastewater, enterovirus was not detected in any of the 9 samples. Fecal coliforms and enterococci were present in significantly higher concentration at Course A. Among 378 whitewater canoeists, gastrointestinal symptoms were 2.97 (95% confidence interval 2.01, 4.37) times more common among canoeists at Course A than at Course B. Beyond that descriptive and compelling information, statistical tests of associations between enteric virus concentration and illness risk were not reported.

Haile 1999[47]: At three beaches in Santa Monica Bay, California 3,554 participants were enrolled in a cohort study of symptom incidence following swimming. Rates of gastrointestinal symptoms were not significantly higher on days that enterococci, fecal coliforms, or total coliforms were elevated. Rates of symptoms were higher when viable human enteric virus was present in the water (based on viral culture) than when viruses were absent, but this did not reach statistical significance at a $p<0.05$ level. The odds of 'highly credible gastrointestinal illness definition 1' were increased (relative to when enteric virus was not detected), with the odds ratio (95% confidence interval) of 1.69 (0.95, 3.01). Based on definition 2 of 'highly credible gastrointestinal illness', the odds ratio was 2.32 (0.91, 5.88).

Conclusions

Based on the studies reviewed, the following answers are provided to the charge questions:

1. **Is there a relationship between male specific and/or somatic coliphage with enteric viruses in recreational waters?**
   As summarized in Table 1, five medium-to-large studies and one small study of coliphages analyzed enteric viral pathogens using culture methods, which identifies infectious or viable viruses. In two of these studies (Skraber, 2004 and Choi 2005), no infectious enteric viruses grew in culture. In two other studies (Moce-Llivina 2005 and Lodder 2010) associations between coliphages and enteric viruses were noted. In one study (Hot 2003) coliphages were not associated with a culturable viral pathogen. These inconsistencies may be due to differences of study settings, virus analysis methods, and fecal pollution sources. A larger number of studies that found no association between coliphages and viral nucleic acids found in water samples (but not necessarily infectious viruses). Taken together, the studies conducted to date provide at best very limited and inconsistent support for an association between coliphages and enteric viruses.

2. **Is there a relationship between male specific and/or somatic coliphage with human health in recreational waters? If so, what is that relationship?**
   As summarized in Table 2, several studies noted statistically significant associations between coliphages and health risk (or suggested associations with 'borderline' statistical significance). Of the four studies that found significant value in coliphage measures as predictors of illness, three also found fecal indicator bacteria to be predictive of illness. One study (Lee, 1997) found coliphage to be a better predictor of health risk than fecal indicator bacteria. However, that study was not conducted in a surface water, but rather at a concrete whitewater slalom course fed partly by wastewater. One study found that fecal indicator bacteria were predictive of illness while coliphage levels were not (van Asperen, 1998). Three studies found that neither fecal indicator bacteria nor coliphages were significant predictors of illness (Von Shirnding, 1992; Abdelzaher, 2011; Dorevitch, 2015). Given the limited number of studies that evaluated coliphages as predictors of health risk and the conflicting findings of those studies, further research is needed before a coliphage-health risk relationship could be characterized.

3. **Is there a relationship between enteric viruses and human health in recreational waters? If so, what is that relationship?**
   As summarized in Table **5**, five cohort studies of water recreation have evaluated enteric viruses as predictors of illness. These studies provide little evidence for an association between enteric viruses and illness among water recreators.

4. **Do any of these papers link coliphage or viruses originating from wastewater that is discharged by centralized facilities to human health? If so, what is the nature of this link and what are the circumstances characterizing the link?**
   As summarized in Table 2, several epidemiologic studies were conducted, at least in part, at sites that were thought to be impacted wastewater discharged by centralized facilities. In one study (Lee, 1997) the whitewater course was partly fed by wastewater. In another (Dorevitch, 2015), one groups of study settings were mainly secondary-treated wastewater. These two studies, neither of which included swimmers, generated conflicting results regarding coliphages as a predictor of health risks (Lee found a strong association, Dorevitch found no association at

the effluent-dominated waters). In the study by van Asperen, no association between illness and coliphage was observed in a setting impacted by treated domestic sewage. In another study (Wiedenmann, 2006) some sites were impacted by wastewater or sewer overflows some of the time. However, the data were not analyzed in a way that would allow the evaluation of whether the observed association between coliphages and health risks differed between the wastewater impacted and non-impacted sites. Finally, the NEEAR study marine sites (Wade, 2010) was conducted at beaches that were within 7 miles of wastewater treatment plants. Coliphages were found to be of some value in predicting illness in that study. On the other hand at two beaches that did not receive treated wastewater, coliphages were found to be predictive of illness at one (Colford, 2007) but not at the other (Abdelhazer, 2011). Thus, no consistent linkage exists between coliphages and illness at wastewater impacted beaches (or at non-impacted beaches).

5. **Are there other recreational water studies not referenced by EPA that evaluate each of the relationships above and meet current conventional standards for epidemiological study? Do these studies change the response to the questions above, and if so, how and why?**
One epidemiologic study (Dorevitch, 2015) was published after the EPA review was released. That study found no predictive value of coliphage at effluent-dominated waters but suggested weak associations between coliphages and illness at other waters during dry weather only. The inclusion of that study does not have a major impact on the overall conclusion that the current epidemiologic literature provides limited and conflicting evidence for coliphages as predictors of health risk.

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