BC Centre for Disease Control
An agency of the Provincial Health Services Authority

Literature review of environmental factors and major sources of sewage affecting norovirus

Prepared for the Environmental Transmission of Norovirus into Oysters Working Group
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Search terms for literature review

Round 1- April 2017

EBSCO Search terms	Results #
Norovir* AND environment* AND transmission	661
Norovir* AND hydrolog*	11
Norovir* AND ocean AND oyster	10
Climate change AND norovir*	19
Water pollution AND norovir* AND oyster	8

Web of Science Search terms	Results #
Norovir* AND environment* AND transmission	105
Norovir* AND hydrolog*	8
Norovir* AND ocean AND oyster	1
Climate change AND norovir*	23
Water pollution AND norovir* AND oyster	13
Norovir* septic	9
Gastro* OR Enteric AND septic AND outbreak	36114
Cruise ship AND norovir* AND discharge	0
Ship AND norovir* AND sewage	1
Ship AND norovir* AND shellfish	6
Waste AND norovir* AND oyster	26
Oyster AND contamination AND sewage	77
Shellfish AND contamination AND sewage	
Norovirus AND seawater	46

Google Scholar Search terms	Results #
Norovir* AND environment* AND transmission	15300
Norovir* AND environment* AND transmission AND oyster	Too many!
Norovir* AND hydrolog*	1100
Norovirus AND hydrology AND oyster	203
Norovir* AND ocean AND oyster	789
Climate change AND norovir* AND water	3360
Water pollution AND norovir* AND oyster	1420
Hydrology sewage pollution marine	39100
Hydrology sewage pollution plume canada	5960
Combined sewer overflow norovirus oyster	235
hydrology of sewage Vancouver plume	772

Round 2- May 2, 2017

EBSCO Search terms	# Results
virus AND metagenom AND [marine OR oyster OR wastewater OR effluent OR sewage]	2
norovirus AND metagenom* AND [marine OR oyster OR wastewater OR effluent OR sewage]	1
norovirus AND metagenom* AND [marine OR oyster OR wastewater OR effluent OR sewage]	0,0,0,0
virus AND metagenom AND [marine OR oyster OR wastewater OR effluent OR sewage]	21
Norovirus AND genogroup AND oyster AND outbreak	32
genogroup AND norovirus AND sewage	112
genogroup AND norovirus AND marine	18
Norovirus AND quantif* AND oyster	23
Norovirus AND geno* AND oyster	131
Indicator AND oyster AND norovirus AND contamination	11
Routine test* AND norovirus AND wastewater OR sewage	1,1
Routine test* AND virus AND sewage	1
Male-specific coliphage AND norovirus AND oyster	2
Male-specific coliphage AND shellfish	5
Male-specific coliphage AND norovirus AND shellfish	3
Male-specific coliphage AND marine animal	0
Male-specific coliphage AND animal	15
Male-specific coliphage AND seasonal*	6
Bacteriophage AND oyster AND norovirus	13
Bacteriophage AND marine animal	5
Bacteriophage AND indicator	792
Microbial source tracking AND oyster	6
Quantitative microbial risk assessment AND oyster AND norovirus	1
Quantitative microbial risk assessment AND virus AND wastewater	118

Web of Science Search terms	# Results
virus AND metagenom AND [marine OR oyster OR wastewater OR effluent OR sewage]	2
norovirus AND metagenom* AND [marine OR oyster OR wastewater OR effluent OR sewage]	1
norovirus AND metagenom* AND [marine OR oyster OR wastewater OR effluent OR sewage]	0
virus AND metagenom AND [marine OR oyster OR wastewater OR effluent OR sewage]	19
Norovirus AND genogroup AND oyster AND outbreak	43
genogroup AND norovirus AND sewage	63
genogroup AND norovirus AND marine	6
Norovirus AND quantif* AND oyster	30

Web of Science Search terms	# Results
Norovirus AND geno* AND oyster	
Indicator AND oyster AND norovirus AND contamination	18
Routine test* AND norovirus AND wastewater OR sewage	1
Routine test* AND virus AND sewage	11
Male-specific coliphage AND norovirus AND oyster	2
Male-specific coliphage AND shellfish	16
Male-specific coliphage AND norovirus AND shellfish	4
Male-specific coliphage AND marine animal	0
Male-specific coliphage AND animal	12
Male-specific coliphage AND seasonal*	6
Bacteriophage AND oyster AND norovirus	13
Bacteriophage AND indicator AND norovirus	19
Microbial source tracking AND oyster	10
Quantitative microbial risk assessment AND oyster AND norovirus	1
Quantitative microbial risk assessment AND virus AND wastewater	51

Table of Contents

Search terms for literature review 3
1. What environmental factors influence the risk of norovirus in oysters? 8
TEMPERATURE 8
HUMIDITY 9
RAINFALL 10
RIVER FLOWS 10
SALINITY 11
SOLAR RADIATION 12
OTHER ENVIRONMENTAL FACTORS 12
Wind and Currents 13
2. Major sources of sewage 14
OVERVIEW 14
SEPTIC TANKS AND OTHER ON-SITE SEWAGE SYSTEMS 17
SEWERAGE NETWORK OVERFLOWS: SSOs, CSOs, and STOs 21
OVERVIEW OF WASTEWATER TREATMENTS 26
WASTEWATER TREATMENT IN CANADA 31
NOROVIRUS AND WWTP 35
DISTANCES TRAVELLED BY NOROVIRUS 41
REFERENCES 50

List of Appendices

Appendix 1. Glossary of terms 43
Appendix 2. Norovirus and other viral outbreaks associated with the consumption of shellfish 45

List of Figures

Figure 1. Possible sources of norovirus contamination related to sewer sytems and waste treatment..... 14
Figure 2. Schematic of septic tank and ground disposal field (image from Ministry of Environment) 17
Figure 3. Combined sewer during normal and overflow conditions. Taken from: http://danieloverbey.blogspot.ca/2014/10/indianapolis-digs-deep-to-fix-its.html 22
Figure 4. Annacis Island WWTP (source: metrovancouver.org) 27
Figure 5. Wastewater Process Flow Diagram. (84) 30
Figure 6. Percentage of the Canadian population served by different levels of municipal wastewater treatment between 1983 and 2009.(93)..... 34
Figure 7. Norovirus densities in raw sewage. Boxplots represent a) different geographical regions; c) region and genogroup; pooled data from all regions is shown for b) sampling season; and d) season and genogroup (diagram from Eftim et al., 2017)(94). 36
Figure 8. Norovirus research in an Edmonton WWTP (top) Treatment stages. (bottom) Virus log removal following municipal wastewater treatment by UV (black) and ultrafiltration (white) treatment trains. Bars represent means ± standard deviation (from Qiu et. al., 2015 (104))..... 38

List of Tables

Table 1. Select outbreaks associated with septic systems 20
Table 2. Outbreaks associated with CSOs, STOs, or SSOs..... 24
Table 3. Research describing CSOs, STOs, or SSOs 25
Table 4: Wastewater treatment classification (45, 87) 29
Table 5. Summary of parameters measured by WWTPs in Canada 33
Table 6. Municipal Effluent Quality Requirements (from table 3 of B.C. Reg. 87/2012)(30) 34
Table 7. Wastewater treatment plants in coastal regions of British Columbia with potential marine impacts 35
Table 8. Log_{10} scale for norovirus genome in water. 35
Table 9. Comparative efficacy between WWTP treatment types for removal of norovirus 42

1. What environmental factors influence the risk of norovirus in oysters?

Many environmental factors can contribute to the survival of norovirus. Key factors identified through a search of relevant scientific literature were cooler water temperatures(1-5), low humidity(6), low salinity(7-9), high rainfall(1, 10, 11) impacting on river flows(12), and seasonality. However, these are not absolute factors that fit every situation. For example, norovirus outbreaks in the southern hemisphere do not always follow the same pattern of winter/cold temperature outbreaks seen in the northern hemisphere(13), and norovirus outbreaks associated with warm water temperatures can be seen in countries such as Mexico(7). Here, further details are given on environmental factors that contribute to norovirus survival in oceanic conditions: temperature, humidity, rainfall, solar radiation (sunlight), and other environmental factors such as gage height and wind currents. How they interact with sewage contamination sources is briefly reviewed.

During the BC norovirus outbreak (Dec 2016 – Mar 2017) average monthly surface air temperatures across Vancouver Island were warmer than average in November (+9°C), and colder than average in December, January, and February (-2°C) (pers. comm. Greg West, EOS UBC). Colder temperatures may have assisted in perpetuating norovirus survival during this outbreak.

TEMPERATURE

Colder water temperatures, typically occurring through late fall and winter, are associated with increased norovirus survival. Temperatures between -6.6 and 20 °C favour the prevalence of norovirus(13). Oysters from waters around England and Wales from temperatures lower than 5°C contained significantly more norovirus compared to oysters from waters 10°C or greater(1). It was suggested that metabolic activity of oysters decreases at colder temperatures, thus slowing virus depuration. Norovirus was detected in oysters up to and including six weeks held at 7 and 15°C under depuration conditions, while at 25°C, norovirus was detected only at two and four weeks. By four weeks, the amount of norovirus present had more than halved from the first week. The explanation given was that “cooler water temperatures extend norovirus clearance time”(2).

In South Korea(14) and Japan(15), a strong negative correlation was observed between temperature and relative humidity, with norovirus survival. Data gathered in England

and Wales showed a relationship between an increase in norovirus (as per laboratory reports) with low temperature over the previous seven weeks, and low relative humidity over the previous five weeks. However, low temperature in the previous week had the strongest effect, with more norovirus cases reported. Further, low population immunity and emergence of new genotypes played a role in increased norovirus reports(16). Murine norovirus (as a surrogate for human norovirus) exposed to monochloramine (a disinfectant) for two hours at 4°C was almost unchanged, but when held at 25°C, norovirus was undetectable after the same period(4).

By contrast, norovirus outbreaks can still occur in warm waters(7), although investigators measured water temperature after transporting samples back to the laboratory, which could have influenced their findings. Nonetheless, temperatures in the two study bays, Altata beach and Mazatlan in Mexico, reach 25 to 31°C and 25 to 29°C in spring, respectively, and range from 32 to 38°C and 30 to 36°C in summer, respectively. Norovirus presence in water was found to decrease as temperatures increased. Thus, cooler water temperatures favour norovirus survivability.

Relative humidity during the BC norovirus outbreak showed a wet November and March, a normal December and February, and a dry January (pers. comm. Greg West, EOS UBC). The dry, cold January seen in 2017 may have played a part in prolonging the norovirus outbreak; however, earlier wet and normal RH observed appear opposite to trends reported in the literature, although not all studies agree on RH in this context.

During the BC norovirus outbreak (Dec 2016 – Mar 2017), a near-record wet November was recorded, while December, January, and February were slightly drier than normal (pers. comm. Greg West, EOS UBC).

HUMIDITY

Humidity affects norovirus infectivity and survival by acting primarily on the capsid structure(6). Humidity is typically measured as either absolute humidity (AH) or relative humidity (RH). AH is a measure of the actual amount of water vapour in an air sample and is typically presented as grams of water vapour/kg of dry air. RH, often given as a percentage, is the ratio of AH to the amount of water vapour that would be needed to saturate the air sample. RH is temperature dependent(6).

As noted above, researchers observed that both low temperature and low RH were associated with an increase in norovirus survival. (14, 16) Contradictory evidence in another study found increased norovirus survival and infectivity when murine norovirus was held at 9°C at both low and high RH (10% and 100%), whereas 9°C at 50% RH was detrimental to survival and infectivity. (6) At a higher

temperature, 25°C, infectious norovirus particles were detected at low (10% and 35%) but not high (55 to 100%) RH. After further data analyses, AH rather than RH appeared to be more critical for norovirus infectivity when AH was below 0.007 kg water/kg air, regardless of temperature, leading the authors to conclude that "winter conditions and especially low AH… corresponding to a temperate climate… provide the ideal conditions for keeping human norovirus infectious…" (6).

RH is an important factor for norovirus survival and transmission, and the majority of studies conclude low RH is important. However, not all studies agree (13), as described in the AH work(6). Further research is necessary to determine the relationship between RH, AH, and norovirus survival.

Extreme rain events trigger storm water and sewage overflows. Continued overflows during the (wet) month of November could have been a key factor in triggering this norovirus event. It would also have increased river flows and decreased salinity, both factors associated with an increase in norovirus contamination, and possibly survival. Conversely, increased rainfall could have increased dilution of norovirus.

RAINFALL

An increase in GII norovirus genotype was observed during rainfall events(10), while rainfall was one of the key factors affecting norovirus contamination in shellfisheries, along with season, and tidal cycle(11).

Contamination of shellfisheries resulted after a concurrent gastroenteritis outbreak in the coastal population and heavy rainfall(17). Rainfall from 1 day to 3 months before an outbreak can favour norovirus prevalence(13). However, in direct contrast to these findings, a study using laboratory surveillance data from England and Wales noted that recent cumulative rainfall was not associated with norovirus incidence(16).No association between rainfall and norovirus was observed in two species of oysters in waters around England and Wales(1).

RIVER FLOWS

Increased river flows are often associated with heavy rainfall events and/or snow melt. High river flows have been associated with an increased risk of norovirus contamination(1, 3, 12). Cumulative river flow in the 7 days prior to sampling was found to be a significant variable contributing to norovirus GI in England and Wales(1).

Reduced solar radiation at the start of the outbreak may have provided one of the conditions allowing this outbreak to begin and continue, although subsequent sunnier than normal months should have counteracted this effect. Sunshine hours during this outbreak could be further investigated.

Other environmental factors associated with norovirus abundance or survival:

- Suspended particulate matter
- Human population density
- Sewage content
- Gage height
- Water mixing
- Wind and currents
- Tidal cycles

Factors NOT associated with norovirus:

- Agricultural sources
- Tidal flows

Similarly, norovirus outbreaks in Lake Ontario, Toronto, were associated with both cold lake temperatures (< 4°C) and high flows in the previous 1 to 7 days in the Don River(3).

SALINITY

To create a model to predict oyster norovirus outbreaks, 21 years of norovirus and environmental data collected from Louisiana oyster harvesting areas along the Gulf of Mexico coast, USA, was used(5). Salinity was included when deciding on what factors to examine in the model because low salinity has been found to enhance virus binding to sediment particles(8). Low salinity was found to be amongst the most important predictive factors of norovirus outbreaks(5). Studies conducted in Mexico(7) and Japan(15) found decreasing salinity favoured norovirus survivability.

Rainfall is clearly a very important factor to consider in norovirus outbreaks – it increases river flows, which would speed the flushing of norovirus to the ocean but also potentially increase dilution; it decreases salinity, which, via various mechanisms, facilitates norovirus survival; and it drives sewage and storm water overflows.

Sewage and storm water overflows occur after heavy rainfalls overload the treatment capacity of the system, sending raw sewage and contaminated water into the aquatic environment (1),(8, 13).

Viruses can bind to fine sediment particles and salinity may enhance this binding process. As a result, viruses are protected by marine sediment and may persist in an infectious state for several months, especially when salinity is low(8).

Heavy rainfalls may then re-suspend sediments, or wash sediments and associated virus particles down rivers and into the ocean(13). Consequently, rainfall can be an important factor causing norovirus release into the environment.

Inferred solar radiation during the BC norovirus outbreak, as per other weather conditions at the time, showed a very cloudy October, November and March, and slightly sunnier than normal December, January, February (pers. comm. Greg West, EOS UBC).

SOLAR RADIATION

Sunlight, or more specifically, solar UV radiation, is a natural virucidal agent in the outdoor environment, killing viruses by chemically modifying their DNA and RNA. Sunlight radiation is the main factor for viral reduction(13). UV can reduce the number of murine norovirus particles, lending weight to the idea that exposure to sunlight kills norovirus(18). Using a 36W lamp, after 120 hours of UV disinfection, murine NOV showed a more than 3 $\log_{10}$ reduction, and infectious murine NoV was not detected after only 72 hours of UV disinfection. However, limitations to UV treatment are turbidity and dissolved salts(18).

OTHER ENVIRONMENTAL FACTORS

Numerous other factors can potentially play a role in norovirus contamination and outbreaks. For example, the combination of light intensity, water mixing, sewage content, and suspended particulate matter determine the abundance and distribution of fecal indicator organisms in the marine environment(9). In studying norovirus in oysters in England and Wales, human population density in the catchment area was positively associated with norovirus, while the combined volume of continuous sewage discharges from the catchment was a predictive factor for total norovirus (GI + GII)(1).

Extremely low gage height (i.e., depth of oyster beds), together with low temperature, low salinity, increased rainfall on the 9th day before an outbreak, and a strong offshore wind, were all associated with increases in (model-predicted probabilities) of norovirus outbreaks(5). Low gage height is essentially low water depth, and may result in a decrease in dilution of virus particles coming from sewage contaminated waters. Heavy rainfall causing sewage system failures and discharge of untreated sewage has been discussed previously, and would potentially allow winds to carry virus particles to oyster beds. This was the only study reviewed here that mentioned gage height as an important factor in oyster norovirus outbreaks, and it was also deemed the most important of the factors in the model.

Average transit times for Salish Sea waters are e.g., Vitoria to Tofino, approximately 2 weeks; Fraser River to Victoria, approximately weeks; and Iona to the Northern Strait of Georgia, in the vicinity of months. The Fraser River flow drives most of the circulation in the Salish Sea, but nothing unusual in circulation or oceanic conditions was observed around the outbreak (pers. comm. Rich Pawlowicz, EOS UBC) i.e., wind directions and currents were observed to be within normal limits.

The role of human-made features in norovirus outbreaks in South Korea found that small-scale, low-tech local sewage treatment plants i.e., discharge of poorly treated sewage, and winter sport areas, specifically ski resorts, where high densities of people accumulate in a small area, were statistically significant factors favouring norovirus outbreaks(19).

In regards to the Salish Sea and local BC conditions, long distance transport of norovirus was deemed possible but unlikely by one knowledge expert (pers. comm. Rich Pawlowicz, EOS UBC).

However, some factors have been dismissed in the literature and are not regarded as important in norovirus contamination. For example, agricultural sources were considered unlikely to be a significant risk for human norovirus contamination (GI and GII)(1). Tidal flows did not affect norovirus levels in oysters(12). By contrast, tidal cycle, along with rainfall and season, was one of the key factors affecting norovirus contamination in shellfisheries(11).

WIND AND CURRENTS

Local wind and current conditions can facilitate norovirus dispersal and distance travelled. However, these conditions are very localized and it is hard to make generalizations from one study area to another because of this variation. A recent review paper noted there is a “significant lack of information about the role of wind in influencing norovirus infections”(13). One study did model both wind speed and direction as an environmental predictor and found onshore winds cause water levels to rise at the coast, and offshore winds cause water levels to fall. Wind was found to be one of the top three predictors of norovirus outbreaks, after gage height and water temperature(5).

Locally, dominant winds in the Strait of Georgia and the Juan de Fuca blow from the northwest in summer and the southeast in winter. Northwesterlies blow along the coast of Vancouver Island in summer, moving coastal waters offshore and lowering the sea level. In winter, winds blow in the opposite direction, moving water onshore and causing

sea levels to rise(20). One hypothesis that could explain onshore winds driving a norovirus outbreak is that coastal contamination is blown back to shore and over shellfish farms, rather than being carried out to sea and diluted.

2. Major sources of sewage

OVERVIEW

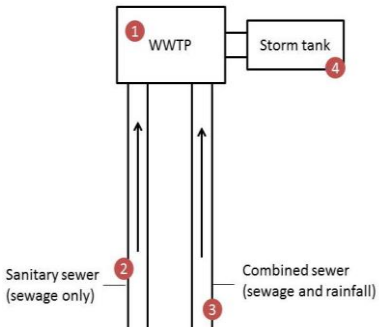
How does sewage enter the marine environment? How is norovirus transmitted from infected humans to the ocean and shellfish farms? Infected individuals can shed up to 10^9 virus copies per gram of feces (21), while asymptomatic individuals can shed virus for over a month (22). An infectious dose is as little as 18 virus particles (23). Thus, even a small number of norovirus-infected individuals can contaminate sewage systems and in turn contaminate shellfish, rapidly transmitting norovirus (24) (25).

Most fresh and marine waters are vulnerable to sewage pollution from various sources depending on geography, population density, regulations governing sewage management, and sewerage infrastructure. Discharges of sewage can enter the marine environment directly or may be introduced into water systems upstream before eventually draining into the ocean. Sewage contamination sources are often categorized as:

Point source: treated effluent from municipal and industrial wastewater treatment plants (WWTP) discharged into the environment via outfall pipes;

Non-point source: municipal drainage works, urban and agricultural runoff, discharges to ground from on-site sewage systems (normally septic tanks), and discharges from vessels(26).

Other sources may contribute to both point or non-point contamination, such as combined sewer overflows (CSOs), sanitary sewer overflows (SSOs), and storm tank overflows (STOs) (27) (Figure 1). These sources will be defined and discussed in this review.



1. Inadequate norovirus inactivation by WWTP
2. Overflow of sanitary sewer (SSO)
3. Overflow of combined sewer (CSO)
4. Overflow of storm tank (STO)

Figure 1. Possible sources of norovirus contamination related to sewer sytems and waste treatment.

The rate at which contaminants enter the environment can be increased by large rainfall events and physical damage to, or faulty installation of, sewerage infrastructure.

In British Columbia (BC), sewage discharges into the environment are governed by the Environmental Management Act (EMA)(28), which contains the Municipal Sewage Regulation (MSR) (29) and Municipal Wastewater Regulation (30). These regulations are informed by the Canada-wide Strategy for the Management of Municipal Wastewater Effluent (31). According to BC legislation, land owners with built structures on the land "must ensure that all domestic sewage originating from the structure[s] is discharged into: i) a public sewer, ii) a holding tank, iii) a sewerage system".

Excess rainfall is a common trigger for sewage-related norovirus outbreaks and other enteric pathogens affecting shellfish and drinking/recreational waters. Runoff can become particularly contaminated with sewage when rainfall occurs following a period of dry weather, during which contaminants build up in stream sediment, storm drains, and other collection points. When rain arrives it triggers a "first flush" into the environment containing a higher-than-normal loading of virus and other pathogens. (8)

A review on the subject of norovirus outbreaks associated with shellfish noted that "the most common route for accidental contamination is sewage overflow and discharge into the aquatic environment during heavy rainfall events" (8).

One of the earliest documented examples of rainfall as a trigger was an outbreak of gastroenteritis in Australia caused by sewage contamination of oyster farms after a period of heavy rainfall. Interestingly, the rainfall was heavy although not unusual, similar to the situation in BC in 2016 (32). Another outbreak in 1982 in New York state associated with oysters and clams was linked to sewage-contaminated coastal waters following heavy rainfall (33). Excess rainfall has been noted during several norovirus outbreaks and instances of contamination in oyster production areas in France (17, 34, 35). One outbreak summary reported: "before the first outbreak, up to 150 mm of rainfall occurred

in less than 1 week, resulting in runoff and river overflow and sewage treatment system failures. Before the second outbreak up to 76 mm of rain fell in 1 day, much more than the monthly average of 65.18 mm"(8). Modeling studies in France have also predicted that fecal contaminant concentrations in tributaries feeding shellfish farms are highest following rainfall events(36).

Runoff from rainfall can contain many infectious pathogens and toxins from multiple sources. Agricultural runoff can contain zoonotic pathogens, while industrial runoff may contain chemical contaminants. Microbial source tracking is a useful tool for determining the origin of sewage, allowing differentiation between pathogens of animal and human sources (37-39). For this review, we are interested in runoff containing human sewage, as this is the main source of human norovirus.

It is not always possible to pinpoint a single cause of oyster farm contamination, because runoff can originate from many sources before arriving at the same marine outfall (40). A study of seven UK oyster catchments found that no single sewage source made up more than 70% of contaminated runoff (41). Thus, contamination events and associated illness outbreaks require detailed localized environmental investigations to conclude whether a particular sewage source is the culprit.

Additionally, experiments using shellfish indicated that repeated lower level exposure to sewage produces a similar level of viral accumulation as a single large exposure, suggesting that shellfish farms near chronic low-level sources of sewage may be similarly at risk as those subject to large contamination events (42). This suggests that ongoing monitoring of shellfish and shellfish water quality near any point or non-point sewage source is essential, although commonly used measurements of fecal bacteria have been found to correlate poorly with norovirus concentration in shellfish tissue (34, 43).

The following sections describe major sources of human sewage as well as any relevant research studies, and descriptions of outbreaks of norovirus and enteric human pathogens related to these specific sources. Throughout,

research and outbreaks concerning shellfish farming are highlighted.

SEPTIC TANKS AND OTHER ON-SITE SEWAGE SYSTEMS

Septic tanks or on-site sewage disposal systems (OSDS) are used in areas that cannot be connected to municipal sewer systems. Septic tanks usually serve a maximum of 150 people. Onsite systems have a tank in which sewage solids collect and settle out, while the remaining liquid flows through a network of perforated pipes into the surrounding disposal field (figure 2).

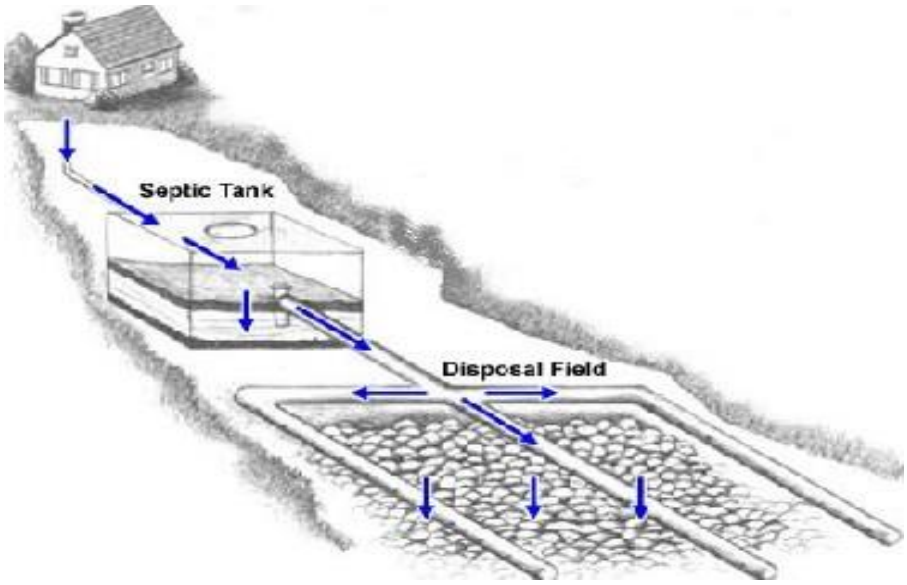


Figure 2. Schematic of septic tank and ground disposal field (image from Ministry of Environment)

During this process, wastewater is filtered by soil, gravel, and naturally-occurring bacteria in the disposal field. There are around 250,000 on-site systems in BC (44). There are various types of on-site systems with different disinfection add-ons that can improve the quality of discharge released into the environment (45).

Holding tanks are watertight containers for storing sewage until it can be pumped out for treatment. These tanks are used in areas were soil type is not appropriate for septic tanks and fields.

Most jurisdictions have a minimum “set-back” distance that an on-site system or holding tank must be placed from ground water sources in order to prevent contamination. In BC, a holding tank must be at least 15 m from a well, and a septic tank must be at least 30 m from a well (section 3.1 of sewerage system regulation of public health act)(46).

Septic systems and illness

The most common way for septic systems to infiltrate the environment and cause illness is via the contamination of groundwater or marine environments where shellfish are grown.

Multivariate analysis found that a higher density of septic systems was associated with viral diarrhea in the United States, where around 25% of households use septic systems. (47). The US Environmental Protection Agency (EPA) designates areas with more than 40 septic systems per square mile as potential ground water contamination zones (48).

The CDC reported that 67% of outbreaks caused by **groundwater** contamination were a result of improperly installed or maintained septic systems (49). A Public Health Agency of Canada meta-analysis of 55 studies found that septic systems were an important contributor to ground water contamination. (50). Sampling of drinking water wells in combination with geographical modeling in Arizona suggested that decay rates of virus (MS-2 coliphage) differed according to factors such as temperature, hydraulic gradient, and soil composition. Researchers found that depending on these factors, certain septic system sites would require set-back distances from ground water as much as 150m. This meant that use of a single recommended set-back distance does not capture real geographic variability (51).

Another study found that increased septic tank density was associated with higher levels of norovirus in the runoff from

streams into the ocean, affecting the risk of swimmers contracting illness (52).

Norovirus and other enteric pathogens have been detected along the Florida coast, associated with septic system seepage into adjacent canals that lead to the Atlantic Ocean (53). It was found that greater density of on-site systems was associated with higher detected levels of enteric pathogens (including viruses). Researchers noted that “the majority of the septic systems in this region…were installed prior to 1983 and do not meet the standards for Florida set-back distances” (54) Further work in the region, employing viral tracers seeded into septic tanks, found that virus could enter adjacent canals and subsequently the wider ocean in a matter of hours (55, 56).There have been various outbreaks of norovirus and other enteric viruses associated with septic systems, summarized in table 1.

Table 1. Select outbreaks associated with septic systems

Pathogen	Transmission vehicle	Country, year	# Sick	Description of trigger and/or contamination source	Citation
Norovirus	Pacific oyster	UK, 2007	NA	Septic tank outfall. 12 months of testing nearby, found virus during every month. Found that currents were carrying contamination along coast with decreasing concentrations from source. Three septic tanks each serving between <10 and 50 people were implicated.	(57)
Norovirus	Well water	United states, 2011	229	A newly installed septic tank, installed in compliance with regulations, was found via viral tracer test to infiltrate the dolomite aquifer well. Trigger: leaking fitting in the septic tank, vulnerable hydrogeological setting	(58)
Norovirus	Groundwater	Iceland, 2013	>100	Septic tank 80 m upstream from a drinking water intake area (48 m was official criteria for se-back distance). Soil composition was too coarse to filter virus from septic effluent and cold temperature favored virus survival. E.coli was filtered out, however.	(59)
Norwalk-like illness	NA	United States	400	“Septic tank effluent” at a resort camp.	(60)
Norovirus	Water storage tank	Shenzhen, China 2009	100s	Underground drinking water reservoir contaminated by septic tank seepage through water source lids.	(61)
Hepatitis A virus	Oyster	Florida, United States	61	Raw oyster consumption, harvesting near failing septic tanks, sewage treatment plant sludge	(62)

SEWERAGE NETWORK OVERFLOWS: SSOs, CSOs, and STOs

Overflows are events in which raw sewage enters the environment via accidental or planned discharges from municipal sewer systems or storm drains. Sewer system overflows are described as either wet or dry weather overflows. Wet weather overflows are a result of excess rainfall or snow melt. Dry weather overflows are any overflows not caused by precipitation, such as sewer line blockages, line breaks, or pumping station power failures (63).

Sewage overflows are most commonly associated with three types of sewer system infrastructure: **sanitary sewers, combined sewers, and storm tanks**. Overflows in these structures are often related to WWTP failure during wet weather, however in this review we will discuss the three overflow types separately from WWTPs.

Sanitary sewer pipes transport sewage from buildings to waste water treatment facilities, and exclusively contain sewage and other waste water. Overflow events associated with these pipe networks are called **sanitary sewer overflows (SSOs)**, and can be either wet or dry weather overflows. In both situations, raw sewage is released into the ground or a body of water before it reaches a water treatment facility. Wet weather SSOs are a result of rainwater inflow through flooding or improperly installed/damaged pipes that exceeds the sewer's capacity, leading to flooding and leakage. Dry weather SSOs can occur when sewer pipes are installed incorrectly or pipes become damaged due to accidents or lack of maintenance. In Metro Vancouver, SSOs in seven sites have automated sampling (64). Monitoring is meant to "inform decisions on potential management options, including collection of data required for design of mitigation infrastructure, if required."

Combined sewers are designed to collect sewage from buildings as well as surface runoff from precipitation. Canadian national standards do not allow new construction of combined sewers, although existing ones may be maintained (31).

When these types of sewers overflow it is called a **combined sewer overflow (CSO)**. Such overflows are almost always wet weather events, especially during storms when the amount of storm water exceeds the capacity of the waste water treatment facility. Combined sewers are designed to discharge excess waste water into the environment under these conditions, similar to a bathtub trap. (26)

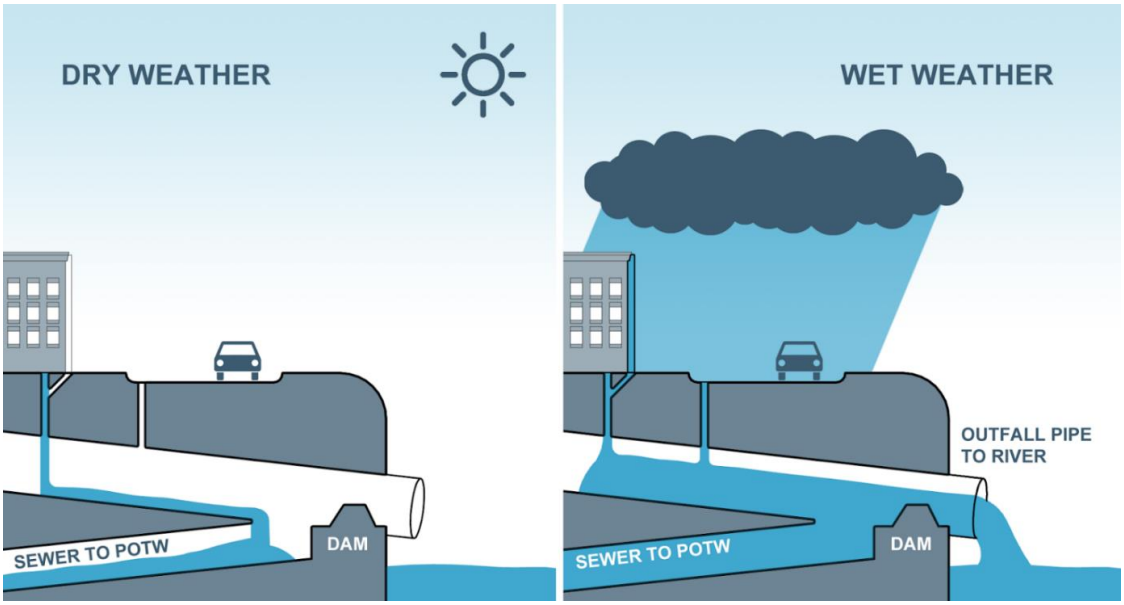


Figure 3. Combined sewer during normal and overflow conditions. Taken from: http://danieloverbey.blogspot.ca/2014/10/indianapolis-digs-deep-to-fix-its.html

Storm tanks are often attached to WWTPs in order to collect excess rainfall runoff or CSO discharge in a separate basin prior to treatment, thus preventing overload of the plant. **Storm tank overflows (STOs)** occur when rainfall exceeds these tanks' capacity.

When CSOs or STOs occur, a combination of storm water and untreated sewage is released, increasing viral load in the receiving environment (65). Several studies investigating the concentration of norovirus throughout sewage treatment plant and adjacent storm overflow tanks found that storm tank discharges represented the main risk of norovirus contamination to local oyster farms (1, 12, 66). Some suggested that the norovirus "risk can be estimated on the basis of the overall volume of sewage discharged into the [shellfish production area] and therefore [norovirus]

contamination will be dependent on the site-specific sewerage discharge arrangements"(1).

More locally, the Greater Vancouver Regional District conducted a watershed-based assessment that looked at the contributions of WWTPs, CSOs, SSOs, and urban storm water runoff, and identified that CSOs have had a "confirmed impact" on local marine embayments (bays), despite being actively phased out (67). This study did not measure norovirus or bacterial pathogens, however combined oxygen demand (COD) was measured, which is an indicator of organic matter in water. In 2014, Metro Vancouver's Liquid Waste Department reported that 20 CSOs operate in the region, which are monitored via the CSO Monitoring Program (64). Using weather forecasting data, the automatic samplers at each site take specimens prior to and during the first flush of wet weather events. Specimens are tested for metals, turbidity, and for microbiological indicators including *E.coli*, enterococci, and fecal coliform. Norovirus is not routinely tested for. The region reported 28.07 million square meters of CSO discharge during 2014 (64).

CSOs can be mitigated by building larger or additional overflow basins and storm tanks to handle excess rainfall, thus improving environmental water quality (68). Adding UV disinfection to storm tanks has been identified as a way to prevent norovirus contamination of oyster beds (66). An extensive study in the UK described sanitary profiles of UK shellfish farm sites before and after sewerage system upgrades such as expansion of storm tanks, removal of combined sewers, and WWTP upgrades, finding that faecal coliform counts were reduced by 39-88% (41).

Existing combined sewers can become prone to overflow as urbanization changes the amount of rainfall runoff entering the sewer. While CSOs are slowly being replaced in many countries including Canada, existing combined sewers are vulnerable to overflow because of increasingly common extreme rainfall events associated with **climate change**. Researchers have identified climate change-induced CSOs as a growing threat to human health via their adverse impact on water quality in shellfish farms (69, 70) and recreational bathing areas (71).

A model built to estimate the impact of CSOs on shellfish farms closures in France found that rainfall events causing a "viral flux from an ill population may reach shellfish beds through raw-water overflows. For the moment this sort of event is still quite rate, occurring only once every 8 years. However, depending on the hydrodynamic conditions, contaminated waters where viruses are present could stay for several days on shellfish beds. If climate change increased the number of extreme rainfall events, this would dramatically lead to long periods of shellfish bed closures" (70). The BC Ministry of Environment advises developers of Liquid Waste Management Plans that "One of the most fundamental issues associated with storm water management is the need to integrate the initial stages of the land use planning process with local watershed hydrology. If land development is undertaken without consideration of watershed hydrology, some of the most important opportunities for minimizing the adverse environmental impacts of the development may be lost" (26)

Table 2. Outbreaks associated with CSOs, STOs, or SSOs

Overflow type	Pathogen or illness	Transmission vehicle	Country, year	# Cases	Description of trigger and/or contamination source	Citation
CSO	Norovirus	Oyster	France, 2017	31	CSO-prone area upstream from six oyster farms with little water outflow. A 28 day closure period was not sufficient to allow depuration of virus from oysters. (had previous outbreaks)	(72)
STO	Hepatitis A	Oyster	France, 2009	111	Outbreak traced to single shellfish farm near a polluted storm sewer.	(73)
SSO	Norovirus	Well water	Sweden, 2001	>200	Sewage pipe malfunction causing overflow and contamination of wells at a recreation facility.	(74)
	Norovirus	Oysters	Australia, 2014	8	A sewer line leaking into nearby oyster farms, confirmed by hydrological study.	(75)

Table 3. Research describing CSOs, STOs, or SSOs

Overflow type	Pathogen or illness	Contaminated item	Country, year	Description of trigger and/or contamination source	Citation
CSO	Norovirus	Oysters, waste water	2013	Found that "a greater percentage (98%) of infectious virus is released in CSO discharges than UV treated effluent (44%). Following a CSO discharge, concentrations of NoV GII and infectious FRNA bacteriophage GA in oysters from less than the limit of detection to 3150 genome copies 100 g^{-1} and 1050 PFU 100 g^{-1} respectively."	(76)
	Faecal indicator bacteria	River water	Paris, 2010	Monitored composition of a CSO discharge following an intense rainfall event triggering a CSO. They estimated that 89% of the CSO came from surface water runoff, and that resuspension of sewage sediment caused by rainfall contributed to the majority of the contamination.	(77)
	NoV and other enteric viruses	Surface waters	Japan, 2014	Rainfall events via CSO lead to increased viral concentration in surface waters.	(10)
	GI illness – ER visits	Drinking water	USA, 2015	Found "an increased risk for GI illness among consumers whose drinking water source may be impacted by CSOs after extreme precipitation."	(78)
	Norovirus	Coastal seawater	Japan, 2004	Followed viral concentrations in receiving environment following a CSO, levels remained the same for four days.	(79)
	E.coli	Oysters and mussels	UK, 2007	Put bags of shellfish in the path of CSOs in intertidal zones. Found that E.coli concentrations increased rapidly following a CSO, regardless of CSO magnitude.	(80)
STO	Norovirus	Oysters	UK	"A positive linear association was found between geometric mean levels of NoV GI + GII in oysters and the number of sewage spills from storm overflows (R^2=74%) at 10 study sites. The model predicted that a NoV concentration of 100 copies/g would correspond to 14	(1)

Overflow type	Pathogen or illness	Contaminated item	Country, year	Description of trigger and/or contamination source	Citation
				sewage spills.	
	Norovirus	Oysters	UK	Characterised oyster contamination in an area frequented by storm water discharges that bypass WWTPs.	(12)
SSO	Norovirus and other viruses	Oysters	France, 2012	Tempest storm damaged sewage pipes (and treatment plants), causing 90% of shellfish farms to be contaminated within 2 days.	(81)
	Norovirus, Hepatitis A, E. coli	Oysters	Australia, 2017	Pump station sewage overflow into oyster harvesting estuary. "NoV GII was detected up to 5.29 km downstream and persisted in oysters for 42 days at the site closest to the overflow."	(82)
	Various enteric pathogens (not NoV)	River water	New Zealand, 2014	Damage to sewers following earthquake leading to raw sewage leakage into river and accumulation in river sediments.	(83)

WASTEWATER TREATMENT PLANTS

OVERVIEW OF WASTEWATER TREATMENTS

Wastewater treatment plants (WWTPs) are designed to remove organic and chemical contaminants in wastewater gathered through sewer and storm pipes. The material entering a WWTP is termed influent, while the water leaving a WWTP is termed effluent.[1] During regular function, influent passes through a screen that removes large solids and debris. The screened wastewater is then subjected to primary, secondary, and/or tertiary treatment depending on the system in place.(45, 84) A video of the Annacis WWTP in Delta, BC illustrates bar screening at the influent pump station removing assorted debris, such as sticks and rags at the beginning of this plant's WWTP process (https://vimeo.com/218315462).(85)

[1] A glossary of key terminology associated with wastewater treatment plants and processes is given in Appendix 1.

Primary treatment involves wastewater being pumped into a large tank where sludge is allowed to settle in purpose-built ponds or tanks, known as waste stabilization ponds, settling ponds, or clarifier tanks. In the Annacis WWTP video, sand and grit is settled out in aerated tanks, followed by removal of fats and oils, which are skimmed from the surface of primary sedimentation tanks.(85) In these tanks, heavy organic material will settle.

Secondary treatment involves the addition of air (oxygen) to wastewater to enhance the ability of bacteria and

Figure 4. Annacis Island WWTP (source: metrovancouver.org)

microorganisms to break down organic matter. Trickling filters are used in the Annacis WWTP. Wastewater is sprayed onto the top of vertical columns of plastic media, the wastewater trickles down the column. The plastic media trays provide over 13,000 square metres of total surface area for bacteria and slime to consume the suspended organic matter that did not settle out in the primary tanks. This activity occurs within four plastic domed areas of the plant (Figure 4).(85) This more recent type of secondary treatment is known as membrane bioreactor technology,

whereby a fine screen or film is used as a frame for bacteria, algae, and fungi to grow upon. Membrane bioreactor technology is increasingly used in WWTPs because it is easily added during plant retrofitting.(86)

Wastewater lagoons are also commonly used to improve water quality during the wastewater treatment process. When properly used, they are considered equivalent to secondary treatment, although cold climates reduce their effectiveness.(45) They are often used in smaller communities, and remove organics and nitrogen loads from wastewater. Following secondary treatment, some plants have **advanced or tertiary treatment processes** that remove viruses, bacteria, phosphorous, ammonia and other dissolved compounds using various chemical and/or biological techniques. The most common tertiary treatments include disinfection via exposure to ultraviolet light (UV), chlorine, and ozone.(45) Ozone and UV are preferred over chlorine as dechlorination is required for effluent discharging to waters containing aquatic life.(87) An overview of the types of WWTP treatments are given in Table 4 and Figure 5.

Sludge formation occurs during primary and secondary treatments. Primary sludge consists of settled solids. Activated sludge is wastewater with bacteria and other microorganisms in suspension. These remove organics, ammonium, and nitrogen from the water and solids as part of the wastewater treatment process.(45) Some WWTPs add flocculation agents to the activated sludge to trap or attract particulate matter in clumps, called wooly-looking masses, or floc, which can then be removed. An important component of sludge treatment is to reduce the water content and ultimately the cost of disposal. Sludge is commonly disposed of via land application e.g., spread on agricultural land, forests, and/or land reclamation sites. Sludge undergoes treatment such as digestion, composting, heating, or other methods in order to reduce microbial loads and remove water. Dewatered sludge can also be disposed of in landfills or burnt in incinerators.

After treatment, effluent from coastal communities may be discharged into seawater via outfall pipes, while effluent from non-coastal communities is often discharged into streams, rivers, or lakes.

Table 4: Wastewater treatment classification (45, 88)

Stage / type of treatment	Treatment	Type of treatment
Primary Mainly physical removal processes	Preliminary	Coarse solids/debris removed with screen before waste water enters settling tank where grit and sand is removed.
	Sedimentation	Suspended solids partly removed by settling out, chemical coagulation, or filtration.
Secondary Mainly biological removal processes	Attached growth processes/fixed film	Tricking filters, biotowers, biological contactors provide a growth medium for bacteria, algae, and fungi to grow and consume organic matter in the waste water.
	Suspended growth processes (activated sludge)	Aerobic bacteria break down organic matter in aeration tanks for several hours. As bacteria accumulate and grow they are removed as "activated sludge"
Lagoons	Lagoons/ treatment ponds	3'to 5' deep, allowing sunlight, bacteria, and oxygen to work together to remove organic matter. Lagoons alone are considered equivalent to secondary treatment.
Disinfection treatments Chemical removal of microbes (bacteria and virus)	Used with secondary & tertiary treatments	Disinfection treatments by chlorine, UV, ozone are often used to supplement primary and secondary WWTP. They may also be used in advanced tertiary treatments.
	Chlorine	Destroys cellular material of microorganisms. Chlorine residue by-product remains in water post treatment, so de-chlorination is often required.
	UV	Leaves no by-product. Damages genetic material in microbes, so they cannot reproduce. Virus may be detectable, but not infective.
	Ozone	Produces fewer by-products than chlorine. Effective at destroying bacteria and viruses.
Tertiary or Advanced Treatments for discharge to fragile ecosystems Lagoons or wetland constructions, physical methods further remove phosphorous, nitrogen and BOD	Nitrification via lagoons, wetlands	Additional biological treatment to allow nitrifying bacteria to convert ammonia to non-toxic nitrate.
	De-nitrification (e.g., Modified Ludzack-Ettinger)	Nitrates are converted to nitrogen gas by methane bacteria in anoxic (zero oxygen) environment. Important in areas where excess nitrate in effluent causes algae blooms.
	Coagulation - Sedimentation	Alum, lime or iron salts are added to remove phosphorous.
	Physical separation methods	Ion exchange, reverse osmosis, membrane filtration and other techniques are used to further polish waste-water and remove dissolved contaminants, organic pollutants, heavy metals, and remaining bacteria and virus.

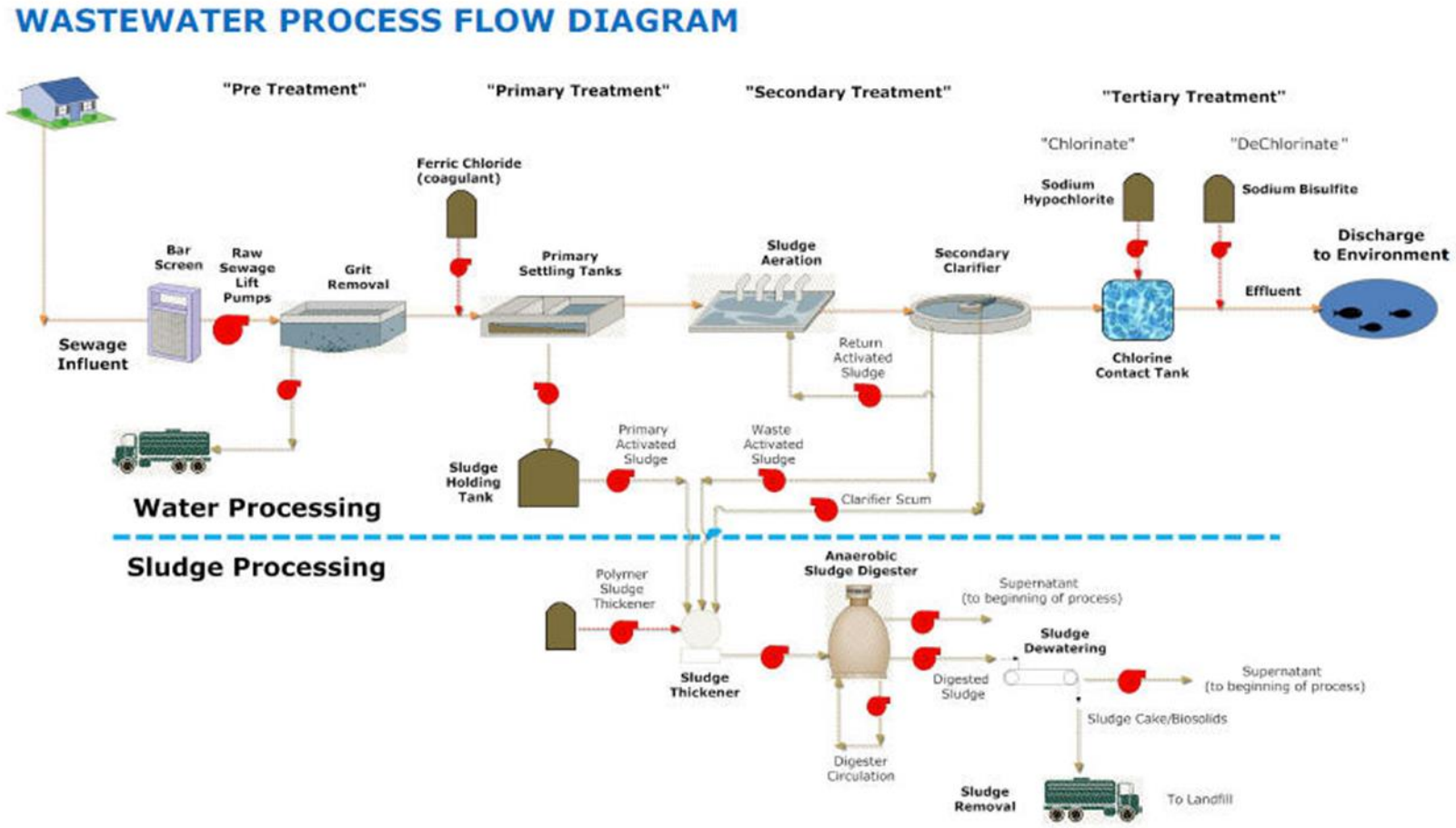


Figure 5. Wastewater Process Flow Diagram. (84)

For example, the discharge pipe in the Victoria outfall is 1.8 km long. For smaller systems, tile fields can also be used as discharge points.

CSOs

Some WWTPs receive both wastewater and storm water. The infrastructure associated with these types of plants is called Combined Sewers (CS). Combined sewer systems can be overwhelmed by storm water, which can result in waste and storm water by-passing the water treatment plant without receiving treatment before discharging directly to the environment. This type of scenario is a Combined Sewer Overflow (CSO), as described in sewerage network overflows, covered in the previous section.

WASTEWATER TREATMENT IN CANADA

Federal Regulations

Federally regulated WWTPs follow the Wastewater Systems Effluent Regulations (SOR/2012-139).(89) Federal regulations cover two types of WWTP – intermittent effluent discharge and continuous effluent discharge. In both cases, the WWTP must have monitoring systems in place to measure either the volume of wastewater or the rate of flow. The composition of the wastewater at the point of effluent discharge are sampled via grab samples, with sampling frequency varying from once every two weeks, to three days per week, depending on the discharge volume. Testing effluent discharge involves providing samples to a laboratory accredited by the International Organization for Standardization and the Environment Quality Act. All reports received from the laboratory must be kept along with WWTP records recording the days when CSO events occurred. A CSO report must be compiled and submitted annually to the authorization officer. The report must state the volume or estimated volume, and number of days in each month during which effluent was discharged through CSOs. All records and reports must be kept for five years.

Municipal Regulations in BC

The regulations that govern municipality wastewater treatment for BC are found in the Municipal Wastewater Regulation under the Environmental Management Act (B.C.

Reg. 87/2002).(30) Municipal regulations overlap, but also go beyond the Federal regulations .

Municipal regulations cover registration of the WWTP, which includes providing details for a local contact person, the name of the WWTP operator, the address of the WWTP, and technical information regarding the WWTP. One section deals with environmental impact studies for WWTPs, including how they should be conducted, by whom, and what should be monitored.

General operating plans, security and assurance plans (in the event the WWTP needs to be replaced), and component and reliability requirements for wastewater facilities are some of the additional regulations within the act, which identify exactly what one would need in order to build and run a WWTP in BC.

Guidelines for what and how often measurements should be taken at WWTPs have been set at the Federal and Municipal level and are summarized in Table 2, while effluent requirements from the BC Municipal Wastewater Regulation are given in Table 3.(30, 89). Many WWTP routinely test effluent samples for bacteria and indicators for viruses, but never for noroviruses. For example, at the Nanaimo WWTP, weekly samples are taken for coliforms and enterococci.(90) Other WWTP are sometimes required under their permit to test for coliforms if they are discharging to a sensitive receiving environment.(90) Biological oxygen demand (BOD), total suspended solids (TSS), fecal coliforms, turbidity and nitrogen amounts are the standard parameters used to measure effluent water.

Table 5. Summary of parameters measured by WWTPs in Canada

Government	Parameter	Frequency and level
Federal	Carbonaceous biochemical oxygen demand (CBOD)	Average taken annually, quarterly, or monthly depending on volume. Taken when discharging effluent. Levels should not exceed 25mg/L.
	Suspended solids	Average taken annually, quarterly, or monthly depending on volume. Taken when discharging effluent. Levels should not exceed 25mg/L.
	Chlorine	Average taken annually, quarterly, or monthly depending on volume. Taken when discharging effluent. Levels should not exceed 0.02mg/L.
	Un-ionized ammonia	Average taken annually, quarterly, or monthly depending on volume. Taken when discharging effluent. Levels should not exceed 1.25mg/L at 15°C ± 1°C.
	Average daily volume	Volume of effluent reported in m^3.
	Influent and effluent	Continuous (equipment must be calibrated annually and at least five months after the last calibration).
	Acute lethality testing (for protected species, e.g., rainbow trout)	Quarterly or monthly depending on average daily volume.
Municipal	Volume	Municipal effluent discharged and the reclaimed water treated and used for each 24 hour period.
	Toxicity monitoring	Depending on maximum daily flow range (m^3/d) from monthly to once every three years.
	5-day Biochemical Oxygen Demand (BOD_5)	Daily to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	Total Suspended Solids (TSS)	Daily to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	Fecal coliform (MPN/100mL)	Daily to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	Turbidity (NTU)	Daily to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	Nitrogen (mg/L)	Daily to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	pH	Not applicable to monthly depending on volume, WWTP classification level, and discharge location (ground, water, marine).
	Total phosphorus (P), (mg/L)	Not applicable to monthly depending on volume, WWTP classification level and discharge location (ground, water, marine).
	Ortho phosphate (P), (mg/L)	Not applicable to monthly, depending on volume, WWTP classification level and discharge location (ground, water, marine).

Table 6. Municipal Effluent Quality Requirements (from table 3 of B.C. Reg. 87/2012)(30)

Requirement	Class A municipal effluent (advanced treatment with the addition of disinfection and nitrogen reduction)	Class B municipal effluent (advanced treatment)	Class C municipal effluent (secondary treatment)
BOD_5 (mg/L)	10	10	45
TSS (mg/L)	10	10	45
fecal coliform (MPN / 100 mL)	median: 2.2 any sample: 14	400, if maximum daily flow is ≥ 37 m^3/d	n/a
turbidity (NTU)	average: 2 any sample: 5	n/a	n/a
nitrogen (mg/L)	Nitrate-N: 10 total N: 20	n/a	n/a

Description of WWTP practices in Canada, and BC

In 2009, 69% of Canadians had access to municipal sewers with secondary or tertiary treatment (Figure 6). Municipal sewers with primary or no treatment accounted for 18%, and another 13% used household septic systems.(91). By comparison, in 2009 in BC, over 40% of the population was served by a WWTP with primary or no treatment, while 10% of residents did not have even basic wastewater treatment services.(92) This is in contrast to Ontario and Manitoba, where over 95% of services are secondary-mechanical treatment, and Alberta, where 78% of the population is on tertiary-level wastewater treatment.(92)

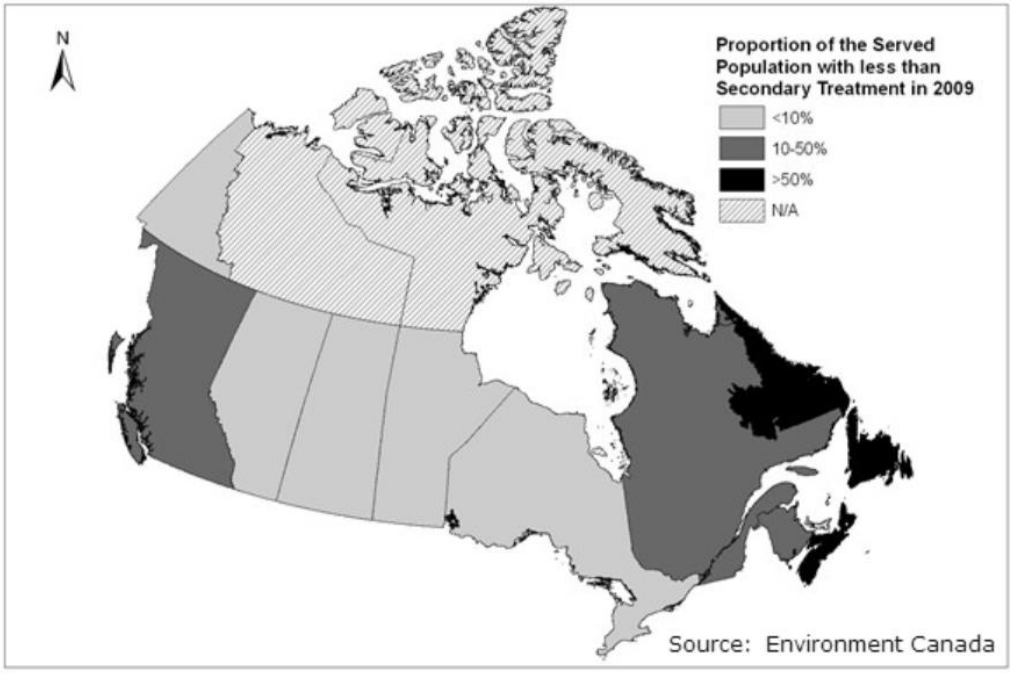


Figure 6. Percentage of the Canadian population served by different levels of municipal wastewater treatment between 1983 and 2009.(93)

In coastal areas of BC, there are at least 93 WWTPs operating that may impact marine waters as of May 2017 (Table 7). Of these, 81% use primary (7), secondary (66) or tertiary (2) treatment, while 18 (19%) do not treat wastewater before discharging into water bodies. Twenty other communities and areas along coastal BC exclusively use septic tank systems connected to outfalls that discharge to marine environments.

Table 7. Wastewater treatment plants in coastal regions of British Columbia with potential marine impacts

Wastewater Treatment Plants with Potential Marine Impacts in Coastal British Columbia					
Treatment type	Total	Disinfection type			
		Chlorine	UV	None	Other or Unknown
No treatment	18	0	0	18	0
Primary treatment only	7	2	0	4	1
Secondary treatment: lagoon	16	1	1	14	0
Secondary treatment: mechanical	50	6	13	29	2
Tertiary treatment: mechanical	2		2		
TOTALS	93	9	16	65	3

Table 8. Log10 scale for norovirus genome in water.

Log $_{10}$	Genome copies per liter
1	10
2	100
3	1000
4	10000
5	100000
6	1000000

NOROVIRUS AND WWTP

Counting norovirus in water. Norovirus in water is typically reported in $\log_{10}$ genome copies per liter of water, abbreviated as $\log_{10}$ gc/L (Table 8). For example, if 1 liter of water contained 1000 genome copies, a 1 $\log_{10}$ reduction would reduce the number of genome copies by 90% to 100, and a 2 $\log_{10}$ reduction would reduce the number of copies by 99% to 10.

Quantity of norovirus found in influent (raw sewage). Two recent meta-analyses (94, 95) and a mini review (96) examined the fate of norovirus in wastewater and its impact on shellfish contamination. WWTPs influent can be assumed to contain norovirus. In general, norovirus GII is detected at higher levels than norovirus GI.(94, 95)

There is also high variability in the number of genome copies per liter of influent, and these also vary by WWTP. Modelling in a North American meta-analysis by Pouillot et al., 2015 found WWTP influent concentrations of 31 gc/L for norovirus GI [1.5 $\log_{10}$ gc/L; 95% CI 0.4 to 2.4 $\log_{10}$ gc/L], and 7943 gc/L for norovirus GII [3.9 $\log_{10}$ gc/liter; 95% CI 3.5 to 4.3 $\log_{10}$ gcL). (95) However, other studies have found much higher levels. In Sweden, average levels of norovirus GI were 6.2 $\log_{10}$ gc/L and GII 6.8 $\log_{10}$ gc/L (97); in the UK, norovirus GI was 2.7 $\log_{10}$ gc/L and norovirus GII 3.6 $\log_{10}$ gc/L (98); and in the most recent global meta-analysis by Eftim et al., 2017, norovirus GI was 4.4 log10 gc/L and GII was 4.9 log10 gc/L (94). Norovirus quantities found in different regions of the world, found seasonally (pooled from all regions), are shown below in Figure 7 from this reference.(94)

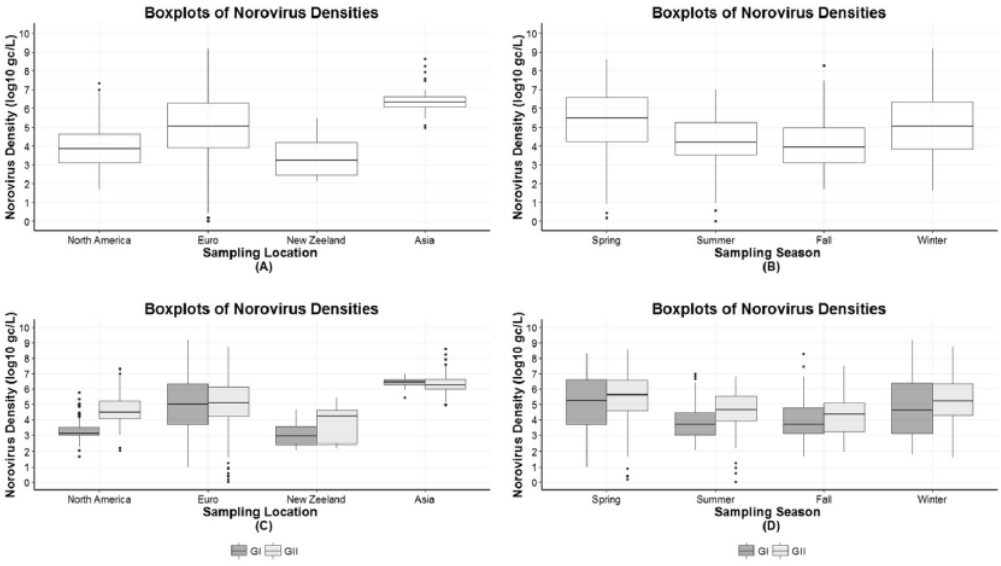


Figure 7. Norovirus densities in raw sewage. Boxplots represent a) different geographical regions; c) region and genogroup; pooled data from all regions is shown for b) sampling season; and d) season and genogroup (diagram from Eftim et al., 2017)(94).

One study found viral concentrations in influent to be independent of community size, although more variability in quantities of norovirus and other viruses were found in smaller communities. Similar norovirus concentrations

between influent and effluent (between 2 and 5 log_{10} gc/L) were found. (99) Higher concentrations of norovirus are found seasonally in the colder winter months in the northern hemisphere.(94, 97, 100)

WWTP removal of norovirus. The effectiveness of wastewater treatment processes in removing norovirus is highly variable (95) and can be influenced by the amount of water entering the system, the amount of norovirus in the influent and the type of wastewater treatment.(98) For example, during rainfall events, wastewater moves at a greater velocity than normal through WWTPs, so it spends less time undergoing treatment.(95) While at least one study found levels of norovirus in both the influent and effluent similar (99), the meta-analysis found WWTP with mechanical systems and chlorine disinfection had higher overall log reductions in viral load than WWTP with lagoon systems and chlorine disinfection.(95) A Swedish study found norovirus reductions was low in several WWTPs, between 0.4 and 1.0 log_{10} gc/L, although the WWTPs were not described.(97) A newly commissioned WWTP in Ireland with secondary treatment and UV disinfection similarly found low overall removal. Norovirus levels were reduced by 0.25 to 0.41 log_{10} gc/100mL in effluent discharging to a shellfish growing area.(76) A secondary treatment WWTP with activated sludge and chlorination in New Orleans, Louisiana also reported a <1.0 log_{10} removal of norovirus GI, which was lower than the removal of norovirus GII (1.4 log_{10}).(101)

During storm conditions (estimated at >10mm rainfall per 24 hours) (36) storm and wastewater can bypass wastewater treatment in two ways. Firstly, it may be released through CSOs distributed at various points along the sewer pipe network without ever reaching a WWTP. Secondly, it may reach the WWTP but be channeled into overflow tanks or pipes and released into water bodies without treatment (treatment bypass). Both types of overflow have been shown to increase the level of norovirus in effluent and shellfish.(10, 96, 102) Typically, the infection risk downstream of WWTPs is considered to be higher than downstream of CSOs largely due to dilution effects (71). Nonetheless, average levels of norovirus in the proximity of CSO outfalls can increase up to 10 times during wet weather.(71) Shellfish collected near discharging CSO outfalls may contain average norovirus levels of 1000 PCR units/g oyster tissue.(96) Even without overflow events, higher water flow through WWTPs decreases waste water transit time, thus treatment may be

less effective in removing norovirus. Of significance to vulnerable shellfish growing areas, one study found the levels of norovirus in settled storm tank wastewater was equivalent to loading from raw influent waters.(103)

Norovirus research in Canada

A Canadian study examined removal of norovirus and other viruses through various waste-water treatment processes (primary sedimentation, secondary treatment, UV disinfection, membrane ultrafiltration, chlorination) at a large WWTP in Edmonton, Alberta.(104) Typical levels of norovirus (5·85 ± 1·05 $\log_{10}$ gc/L) in 16 influent samples were observed.(95) Removal of viral DNA, including norovirus, detectable by PCR, was much greater after membrane ultrafiltration compared to using UV disinfection (Figure 5). Culture of viruses other than norovirus demonstrated a 30% decrease in viral infectivity during UV treatment.

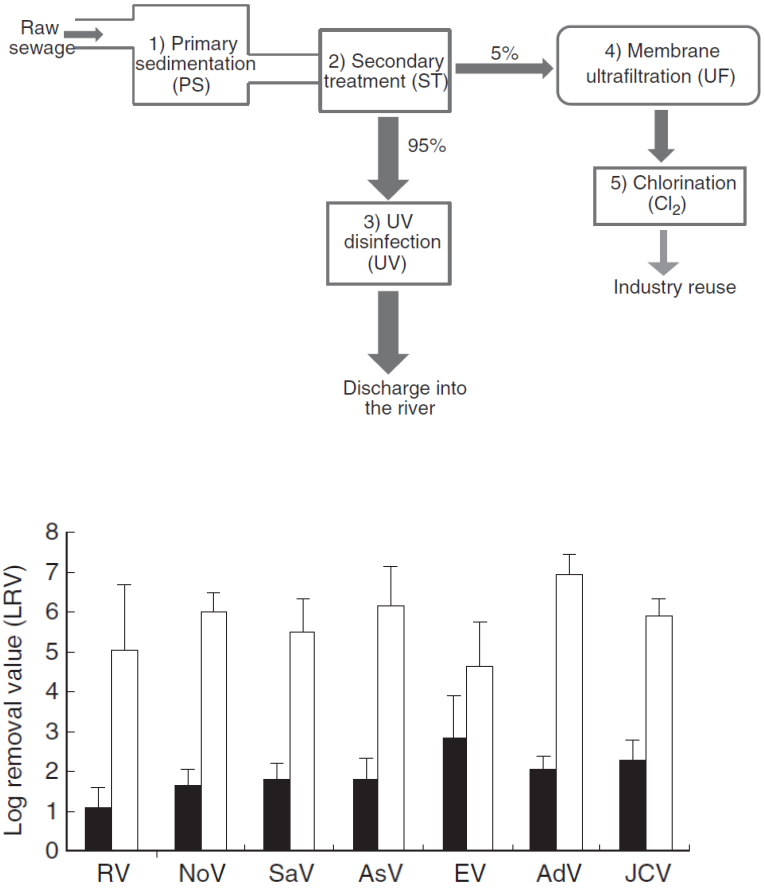


Figure 8. Norovirus research in an Edmonton WWTP

(top) Treatment stages.

(bottom) Virus log removal following municipal wastewater treatment by UV (black) and ultrafiltration (white) treatment trains. Bars represent means ± standard deviation (from Qiu et. al., 2015 (104)).

Norovirus research elsewhere

In a study of five WWTPs in the UK, primary treatment using settlement tanks resulted in an average 1 log_{10} reduction in norovirus, with secondary treatment providing a further 1.5 log_{10} reduction. Reductions were highly variable, ranging from a 6.1 log_{10} reduction in GI to a 6.5 log_{10} reduction for GII when using secondary treatment.(98) Secondary treatment using activated sludge (modified Ludzack-Ettinger) was most effective in removing norovirus, followed by trickling filters, biological aerated filters, and humus tanks.(98) Similarly, following one year of measurement at a single WWTP, a Swedish study found slight reductions from incoming water (1.5log_{10}) to primary settling (0.9 log_{10}) to secondary settling (0.4log_{10}) to trickling filter treatment (0.1log_{10}) through-out treatment.(105) For plants where secondary treatment was partially effective in reducing norovirus, UV treatment and to a lesser extent chlorine treatment, further reduced norovirus levels, but this reduction was dependent upon UV intensity or chlorine concentration.(66, 86)

Membrane bioreactor was found to be more effective than conventional secondary treatments in removal of norovirus GI (median 3.02 log_{10} gc/L over 1.43 log_{10} gc/L) (86). Foam separation resulted in an 8-fold reduction of norovirus in wastewater in one report. (106)

Overall, tertiary UV exposure is considered the most effective method of inactivating viruses, although the Alberta study found membrane ultrafiltration removed higher viral loads than UV treatment. (95, 98, 104) However, UV impact is reduced in the presence of suspended solids, requiring optimization of primary and secondary treatments to increase overall effectiveness.(107) Very few studies have compared the efficacy of norovirus removal from effluent between different types of WWTP designs. Study results are summarized in Table 9.

Limitations

Although PCR tests detect and quantify viral RNA from norovirus (genome copies), it is unknown whether the genetic material is from intact viruses capable of causing human illness, or from non-infective particles. Because of this limitation, there has been interest in using cultivable indicator viruses to more accurately measure removal of

infectious viral particles during WWT processes. The virus used most often as an indicator for norovirus is male-specific coliphage (also called F-specific coliphage, MSC). MSC is a virus whose natural hosts are coliform bacteria that grow in warm environments (i.e., the mammalian intestinal tract) therefore MSC are found in association with mammalian fecal material. MSC are ubiquitous in wastewater, are RNA viruses similar in size and shape to noroviruses, and can be grown in bacterial cell cultures with a short turn-around time (one day or less). Studies using MSC virus have shown that PCR testing underestimates the reduction in infectious particles, and therefore can underestimate the effectiveness of treatment processes including lagoons, UV, and chlorine (76, 95, 99).

Dispersal and persistence of norovirus

There is limited research describing how norovirus particles disperse and persist after discharge of effluent into ocean environments. Norovirus accumulation in mussels follows a tidally-driven effluent dispersal model, which differs in spatial pattern to *E. coli*.(108) In this study, mussels hung in cages around sewage discharge areas showed that while the dispersion did match MIKE21 (model) predictions, coliform and *E.coli* dispersions did not reflect viral dispersions in marine waters. (108)

There appears to be a gradient of norovirus in oysters corresponding to distance from a sewage outflow. In one study in New Zealand, total norovirus levels adjacent to the outfall were about 1,000 PCR units/g oyster, decreasing to 130 PCR units/g at 10 km and 100 PCR units/g at 24 km from the outfall.(96) In another study in Ireland, depending on the local hydrodynamic conditions, the extent of impacted area could be in excess of 4 km from the implicated discharge point.(96) Norovirus GII was found as far as 5.74 km from a discharge point, close to the Food and Drug Administration dilution level of 1,000:1. At one station measured, MSC findings were above the ISSC critical limit of 50 pfu/100 g with positive norovirus findings. At this site a dilution level of 556:1 or less was insufficient to prevent norovirus contamination of oysters.(109)

A study assessing norovirus and *E.coli* levels in oysters placed in proximity to WWTPs found decay rates for norovirus to be lower than those for *E.coli;* norovirus was detected up to 12.3 km away from the WWTP outfall (a geometric mean of 3 log10 gc/gram was found in

oysters).(12) Poorly treated sewage may result in norovirus contamination in shellfish up to 10km or more from a WWTP or sewage discharge site.(12) Norovirus has been reported to persist in oysters for 4 to 6 weeks after a point in time sewage contamination event.(96) Oysters exposed to one large sewage spill and those exposed to smaller, repeated sewage events have been reported to accumulate similar amounts of norovirus (42).

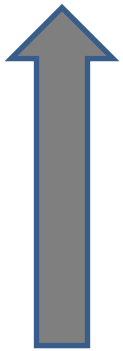
Conclusion

Norovirus is not completely eliminated using best-available WWTP practices in optimally operating systems. Older technology, treatment plant malfunctions, and high rainfall events further increase the amount of norovirus entering the marine environment via wastewater

DISTANCES TRAVELLED BY NOROVIRUS

There are relatively few studies that attempted to measure the distance norovirus can travel. The work done to date has measured norovirus in shellfish indicators (oysters or mussels) at various distances from sewage contamination sites. In most studies, shellfish were not located far enough away from the outfalls and the actual outer limit or boundary zone where norovirus might be found was not established. In one study, the same amounts of norovirus were detected in an outfall as in the oysters located over 7km downstream from the WWTP outfall. By 10km distance from the outfall, norovirus had decreased by only 0.6 $\log_{10}$ in the sampled oysters(12). After a raw sewage overflow during a heavy rain event, where more than 3000 kL of raw sewage was discharged into the coastal environment in New Zealand, norovirus GII was detected in oysters 8.5km distance from the outfall point(12). Various studies are cited which observed that norovirus in the marine environment can be transported greater than 10 km from the discharge point(110). A gradient of norovirus was observed in New Zealand shellfish corresponding to distance from an outfall. At the point of the outfall 1000 PCR units/g were detected in shellfish; at 10 km away, 130 PCR units/g, and at 24 km away 100 PCR units/g (G. Greening).(27)

Table 9. Comparative efficacy between WWTP treatment types for removal of norovirus

Reference	Pouillot et. al., 2015 (95)	Qui et. al., 2015 (104)	Campos et. al., 2016 (98)
	2^o Mechanical + UV disinfection	3^o Membrane ultrafiltration	3^o UV disinfection
	2^o Mechanical + Cl_2 disinfection	3^o UV disinfection	2^o activated sludge
	2^o Lagoon + UV disinfection		2^o trickling filter
	2^o Lagoon + Cl_2 disinfection		2^o biological aerated filters
			2^o humus tanks
Increasing effectiveness of norovirus removal			

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Appendix 1. Glossary of terms (111)

Term	Description
Activated Sludge	Sludge that has undergone flocculation forming a bacterial culture typically carried out in tanks. Can be extended with aeration.
Advanced Primary Treatment	The use of special additives to raw wastewater to cause flocculation or clumping to help settling before the primary treatment such as screening.
Advanced Wastewater Treatment	Any advanced process used above and beyond the defacto typical minimum primary and secondary wastewater treatment.
Clarifier	A piece of wastewater treatment equipment used to "clarify" the wastewater, usually some sort of holding tank that allows settling. Used when solids have a specific gravity greater than 1.
Combined Sewer	A system where the storm drains and sewer drains are combined. The advantage to this is storm water, which can contain contaminants, gets treated.
Combined Sewer Overflow (CSO)	When the amount of liquid entering into a combined sewer overwhelms the system and causes and overflow. Generally resulting in a discharge of unprocessed liquids
Digestion	The breaking down of sludge and other waste biologically by microorganisms. Results in byproducts such as methane gas, carbon dioxide, sludge solids and water. Aerobic digestion requires oxygen, anaerobic digestion the absence of oxygen.
Disinfection	The use of chemicals to kill any disease causing organisms in the polished wastewater. UV light can also be used.
Effluent	Liquids existing a wastewater treatment plant in a treated or untreated state.
Flocculation	The process whereby a chemical or other substance is added to wastewater to trap or attract the particulate suspended solids into clusters or clumps of floc or flocculent, wooly looking masses.
Influent	Liquids entering into a wastewater treatment plant, basin or reservoir. These can include grey water, black water and storm water runoff.
Primary Wastewater Treatment	The first process usually associated with municipal wastewater treatment to remove the large inorganic solids and settle out sand and grit.
Retention time/Residence time	The average amount of time wastewater spends at the wastewater treatment plant. This time can vary greatly due to the types of systems and plants, from minutes to weeks.
Secondary Wastewater Treatment	Second biological process of digestion with bacteria.

Sludge/biosolid	The solid waste that settles to the bottom of the wastewater treatment process. Sludge can be dewatered and reused or disposed of.
Strom Water Run-Off (SRO)	The pulse of surface water following a rainstorm. The water carries sediment, gas, oil, animal feces, glass and other waste from the watershed to receiving waters creating a difficult urban/suburban wastewater problem.
Tertiary Wastewater Treatment (Advanced)	Biological or chemical polishing of wastewater to remove organics, solids and nutrients. Tertiary wastewater effluent limits are generally 10 mg/1 BOD5 and 10 mg/1 TSS.
Tertiary Treatment	The use of filtration to remove microscopic particles from wastewater that has already been treated to a Secondary Level.
Ultraviolet Disinfection	The use of ultraviolet light to kills bacteria and other microorganisms in water and wastewater. Typically a final treatment step.
Wastewater Treatment Plant (WWTP)	A WWTP is designed to treat wastewater from sewer and/or storm water pipes in order to reduce harmful materials.

From: http://www.mgsdistrict.org/wp-content/uploads/2011/11/Glossary-of-Wastewater-Terms.pdf (111)

Appendix 2. Norovirus and other viral outbreaks associated with the consumption of shellfish

Shellfish	Month - Year	Country	No. of cases (outbreaks)	Stool analysis	Shellfish analysis	Comments	Reference
Cockles	Dec-76	England	33	EM NV			(112)
Oysters	Jun-78	Australia	2000	EM NV		Contamination by sewage	(32)
Oysters	1983 to 2014	Global				>80% of all NV genotypes detected in oyster samples and oyster OBs. Difference in types in coastal vs. other communities. Oysters act as vector AND reservoir for human infection	(113)
Oysters	Jan-83	England	137	EM NV			(114)
Oysters	Dec-83	Australia	14	NV GII		Imported frozen Japanese oysters, 3 types of GII in a single sample, potential for recombination discussed	(115)
Oysters	1987-94	Japan	(4)	NV GI	NV GI, GII		(116)
Clam	Jan-88	China	>92,000	HAV	HAV	Epidemic HAV. Estimated 32% of those diagnosed with HAV ate clams, cooked and raw. Contaminated growing area	(117)
Oysters	Aug-88	US	61	HAV	HAV	Illegal harvest from an unapproved area. Night harvesting. Failing septic tanks, boat sewage disposal and sewage treatment next to unapproved areas	(62)
Mussels	1987 to 2007	Italy	Case rates for HAV measured		HAV in 1987: 18% + (n=53 samples). No HAV detected in 2007 (+ for NV, RoV, EV)	Vaccination resulted in drop in HAV case rate. Decreased from 130 per 100,000 in 1997 to 2.4 per 100,000 in 2009 and absence of contamination of mussels in 2007 survey	(118)
Oysters	1993-6	US (LA)	(6)	Snow mountain, Lordsdale, GII.3	SRSV	Overboard disposal of sewage (summary of LA and FL outbreaks)	(119)

Shellfish	Month - Year	Country	No. of cases (outbreaks)	Stool analysis	Shellfish analysis	Comments	Reference
Oysters	Nov-93	US (LA)	73	EM NV, RT-PCR		Oyster harvesters, overboard sewage disposal. People eating well-cooked oysters in stew didn't become ill	(120)
Oysters	Nov-93	US (Florida)	30	EM NV, RT-PCR		Contamination from oyster harvesters	(121)
Oysters	Jan-95	US (Florida)	70	EM NV, RT-PCR		Contamination from oyster harvesters, recreational boaters	(122)
Oysters	1996	Denmark	356	NV, EV	NV, EV	Traced to imported product, possible fraud	(123)
Oysters	Feb-96	US (LA)	72	EM NV, RT-PCR		Contamination from oil rig, oyster harvesters	(119)
Seafood	May-96	Italy	562	HAV		Raw seafood consumption, because of high HAV incidence, control of water recommended	(124)
Oysters	1996-7	US (LA)	153	SRSV		Contamination from oyster harvesters, inadequate sewage onboard vessels	(119, 125)
Oysters	May-98	US (CA)	171	NV	NV	Raw and undercooked oysters. No environmental explanation, suggested boat discharge, malfunctioning sewage disposal systems	(126)
Oysters	1996 to 2009	Osaka City, Japan	(96 of 505 NV OBs from oysters)	4 to 17 genotypes observed each season	multiple strains in 43 oyster OBs (clinical sample)		(125)
Clam	1999	Spain	184	HAV	HAV	Frozen imported coquina clams	(127)
Oysters	Sep-99	New Zealand	86 (10)	NV GI, GII.3	GII.3	93 OBs in total reported during timeframe. Boats near farms allowed treated discharge 100m from farms. Zone around treated sewage is 400m.	(128)

Shellfish	Month - Year	Country	No. of cases (outbreaks)	Stool analysis	Shellfish analysis	Comments	Reference
Oysters	Mar-00	France	14	NV GI	NV GI	Low E.coli counts in shellfish, but NV persisted in harvest for several weeks	(129)
Oysters	2000-2002	Hong Kong	(13)	GI and GII	NV detected in 6 OBs. GI and GII	10% imported oysters positive for NV. Clinical and oyster genetic sequences rarely matched. Oysters dessiminate new strains	(130)
Oysters	Apr 2001 to Jan 2005	Japan	(95 of 290 NV OBs from oysters)	usually a single strain	multiple strains	Attack rate (AR) higher in oyster vs Food Handler (FH) OBs; genogroup differences in AR: GII.4 lower, GII.3 higher (suspect b/c GII.4 causing more of LTCF type OBs, not FH ones)	(131)
Oysters	2001 to 2012	Osaka City, Japan	(88)	NV detected in 69%; also Aiv, AV, SaV, EV, RV A			(132)
Oysters	2002 to 2006	Japan	(11)	NV, KV, SaV, AV (multiple NV, SaV genogroups)			(133)
Mussels	Apr-02	Italy	103	NV	NV GII and GI	Raw and cooked mussels caused illnesses. Samples negative for bacteria (met standards), not reliable indicator for viral contamination	(134)
Oysters	Dec-02	France	127	NV GI.4, GII.4,	GI.4, GII.4, 8	Flooding and sewage treatment plant failure	(35)
Oysters	Dec-02	Italy	202	NV GI.6, 4 GII.4, 8			
Oysters	2003-04	Australia	83	NV GI.4, 2, GII.6, 7, 9, 5, 12	NV GII.4	Imported from Japan, same estuary	(135)
Oysters	Dec-03	Singapore	305	NV GII	EM NV	Imported half-shell frozen oysters from China	(136)
Oysters	Jan-04	Canada	135	NV GI.1, 2, GII. 3, 4, 5	GI.2 and GII	Widespread contamination	(137)

Shellfish	Month - Year	Country	No. of cases (outbreaks)	Stool analysis	Shellfish analysis	Comments	Reference
Mussels, razor shell	Jan-04	Italy	882	HAV	HAV	Breeding farms from various area and illegal storage in seawater	(138)
Oysters	Aug-05	US	39	HAV	HAV	Probable illegal waste discharges from harvest vessels or recreational boats, illegal harvesting in closed areas	(139)
Oysters	Feb-06	France	205	AiV, AV, EV, RV, NV GI, GII (7 NV genotypes)	AiV, AV, RV, NV GI, GII	Flooding and sewage treatment plant failure	(34)
Oysters	Jan-07	Sweden	30	NV GI.1	NV GI.1, GII.3	Inappropriate storage for 10 days in corf sunk in a guest-harbor	(140)
Oysters	Jul-07	France	111	HAV		Polluted storm sewer during depuration in tank	(73)
Oysters	2007-10	UK	315 (12)	NV GI and GII	NV GI and GII	Copy # of NV in non-outbreak areas one log lower (152 cpg)	(141)
Oysters	Feb-08	France	23	NV GII.4, SaV, AiV	NV GI, II.4 SaV	Illegal collection of oyster from a forbidden area	(142)
Clams	Jun-08	Japan	38	NV SaV	NV SaV capsid sequencing similar to humans	Clams (*Ruditapes philippinarum*) in an au gratin dish, virus detected from clam and liquid in package	(143)
Razor clams and Oysters	Feb-09	UK	240	GI and GII.2, 3, 4, 6	NV GI and GII	Same supplier for clams and oysters, depuration and environmental conditions appear normal. FHs also ill, implicating a langoustine cream dish.	(144)
Oysters	Dec-09	US (North Carolina)	177 (37 were secondary cases)	GII.12	ND	Inadequately steamed oysters	(145)
Oysters	Feb-09	Alaska	35	NV GI and GII	NV GII	Oysters hung near boat harbours for 24 hrs before sale	(146)

Shellfish	Month - Year	Country	No. of cases (outbreaks)	Stool analysis	Shellfish analysis	Comments	Reference
Oysters	Jan 2010, Jan 2012	Ireland	88 (2)	NV GI.1, 2, 4, 6, 11. GII.I, 3, 4, 6, 7, 13	NV GI.1, 2, 4, 11 GII.1, 3, 4, 6, 7, 12, 13	Multiple genotypes in harvest area and leftover oysters. WWTP 1 km from oyster growing area, high rainfall on Jan 4, 2012. Oysters depurated. E.coli monitoring unsuitable for virus.	(147)
Oysters	Jan-10	UK, Norway, France, Sweden and Denmark	334 (65, confirmed NV in 22)	NV GI and GII	NV GI and GII	Widespread contamination, oysters from England, Scotland, Ireland, France, Netherlands	(148)
Oysters	Sep-10	Canada	36	NV GI.4	NV GI.4 and GII.4, GII.6/7/9	Ill shellfish harvester, overboard disposal	(149)
Mussels	2012-13	Netherlands	9	HAV GIA		Domestic sewage discharge into production area, HAV acquired from travel to endemic area	(150)
Oysters	Jan-12	France	84	NV GII	NV GII	Same NV GII in production area, illnesses from lunch of shellfish in nursing home	(151)
Oysters	Dec 2012, Jan 2015, Mar 2015	France	31	NV GI and GII	NV GI and GII	Same production area, depuration did not work, high NV GI prevalence in oysters, 28 days is too short for farm closures	(72)
Oysters	Mar-13	Australia	525 illnesses in total. NSW n=8; Tasmania n=306; Victoria and Queensland n=211	GII	GII	Leaking sewer line	(75, 152)
Oysters	May-13	South Korea (Gyeonggi Province)	8	GII.4, GII.11, GII.14	GII.4, GII.11, GII.14	Fermented oysters	(153)
Oysters	Jan-16	Denmark	58	GII.P17-GII.17	GI.2, GII.17	Oysters from France, new Asian NV strain	(154)
Oysters	2017	Canada	331			Multiple harvest areas	(155)

Abbreviations: OB = outbreak; FH = foodhandler; EM = electron microscopy
Viruses: NV = norovirus; HAV = hepatitis A virus; AiV = aichivirus; AV = astrovirus; SaV = sapovirus; EV = enterovirus; RV = rotavirus; KV = kabuvirus; SRSV = small, round, structured-virus (this was how Norovirus was described by EM methods)

REFERENCES

1. Campos CJ, Kershaw S, Morgan OC, Lees DN. Risk factors for norovirus contamination of shellfish water catchments in England and Wales. Int J Food Microbiol. 2017;241:318-24.
2. Choi C, Kingsley DH. Temperature-Dependent Persistence of Human Norovirus Within Oysters (Crassostrea virginica). Food Environ Virol. 2016;8(2):141-7.
3. Greer AL, Drews SJ, Fisman DN. Why "winter" vomiting disease? Seasonality, hydrology, and Norovirus epidemiology in Toronto, Canada. Ecohealth. 2009;6(2):192-9.
4. Keller R, Tetro JA, Susan Springthorpe V, Sattar SA. The Influence of Temperature on Norovirus Inactivation by Monochloramine in Potable Waters: Testing with Murine Norovirus as a Surrogate for Human Norovirus. Food Environ Virol. 2010;2(2):97-100.
5. Wang J, Deng ZQ. Modeling and Prediction of Oyster Norovirus Outbreaks along Gulf of Mexico Coast. Environ Health Perspect. 2016;124(5):627-33.
6. de la Noue AC, Estienney M, Aho S, Perrier-Cornet JM, de Rougemont A, Pothier P, et al. Absolute Humidity Influences the Seasonal Persistence and Infectivity of Human Norovirus. Appl Environ Microbiol. 2014;80(23):7196-205.
7. Felix JL, Fernandez YC, Velarde-Felix JS, Torres BV, Chaidez C. Detection and phylogenetic analysis of hepatitis A virus and norovirus in marine recreational waters of Mexico. J Water Health. 2010;8(2):269-78.
8. Maalouf H, Pommepuy M, Le Guyader FS. Environmental Conditions Leading to Shellfish Contamination and Related Outbreaks. Food Environ Virol. 2010;2(3):136-45.
9. Campos CJA, Kershaw SR, Lee RJ. Environmental Influences on Faecal Indicator Organisms in Coastal Waters and Their Accumulation in Bivalve Shellfish. Estuaries and Coasts. 2013;36(4):834-53.
10. Hata A, Katayama H, Kojima K, Sano S, Kasuga I, Kitajima M, et al. Effects of rainfall events on the occurrence and detection efficiency of viruses in river water impacted by combined sewer overflows. Sci Total Environ. 2014;468:757-63.

11. Lee RJ, Morgan OC. Environmental factors influencing the microbiological contamination of commercially harvested shellfish. Water Science and Technology. 2003;47(3):65-70.
12. Campos CJA, Avant J, Gustar N, Lowther J, Powell A, Stockley L, et al. Fate of Human Noroviruses in Shellfish and Water Impacted by Frequent Sewage Pollution Events. Environ Sci Technol. 2015;49(14):8377-85.
13. Shamkhali Chenar S, Deng Z. Environmental indicators for human norovirus outbreaks. Int J Environ Health Res. 2017;27(1):40-51.
14. Kim YS, Park KH, Chun HS, Choi C, Bahk GJ. Correlations between climatic conditions and foodborne disease. Food Research International. 2015;68:24-30.
15. Takahashi H, Takahashi M, Ohshima C, Izawa Y, Uema M, Kuda T, et al. Differences in the viability of murine norovirus in different aquatic locations. Mar Pollut Bull. 2016;112(1-2):313-7.
16. Lopman B, Armstrong B, Atchison C, Gray JJ. Host, weather and virological factors drive norovirus epidemiology: time-series analysis of laboratory surveillance data in England and Wales. PLoS One. 2009;4(8):e6671.
17. Miossec L, Le Guyader F, Haugarreau L, Pommepuy M. Magnitude of rainfall on viral contamination of the marine environment during gastroenteritis epidemics in human coastal population. Revue D Epidemiologie Et De Sante Publique. 2000;48:62-71.
18. Correa AD, Souza DSM, Moresco V, Kleemann CR, Garcia LAT, Barardi CRM. Stability of human enteric viruses in seawater samples from mollusc depuration tanks coupled with ultraviolet irradiation. J Appl Microbiol. 2012;113(6):1554-63.
19. Kim MS, Koo ES, Choi YS, Kim JY, Yoo CH, Yoon HJ, et al. Distribution of Human Norovirus in the Coastal Waters of South Korea. PLoS One. 2016;11(9):1-17.
20. Davenne E, Masson D. Water properties in the Straits of Georgia and Juan de Fuca. Sidney, BC: Fisheries and Oceans Canada, Sciences IoO; 2001.
21. Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH, et al. Norwalk Virus Shedding after Experimental Human Infection. Emerg Infect Dis. 2008;14(10):1553-7.

22. Leon J, McDaniels M, Lyon GM, Abdulhafid G, Dowd M, Etienne K, et al. Norovirus human infectivity, immunology, and persistence in groundwater. FASEB J. 2008;22.
23. Hall AJ. Noroviruses: The Perfect Human Pathogens? The Journal of Infectious Diseases. 2012;205(11):1622-4.
24. Miura T, Lhomme S, Le Saux JC, Le Mehaute P, Guillois Y, Couturier E, et al. Detection of Hepatitis E Virus in Sewage After an Outbreak on a French Island. Food Environ Virol. 2016;8(3):194-9.
25. Miossec L, Le Guyader F, Haugarreau L, Comps MA, Pommepuy M. Possible relation between a winter epidemic of acute gastroenteritis in France and viral contamination of shellfish. J Shellfish Res. 1998;17(5):1661-4.
26. Interim guidelines for preparing liquid waste management plans. In: Environment BMo, editor. Revised edition ed2011.
27. Campos CJA, Lees DN. Environmental transmission of human noroviruses in shellfish waters. Appl Environ Microbiol. 2014;80(12):3552-61.
28. Environmental Management Act, (2003).
29. Municipal sewage regulation, 129 (1999).
30. Municipal wastewater regulation, (2012).
31. Canadian Council of Ministers of the Environment. Canada-wide Strategy for the Management of Municipal Wastewater Effluent Whitehorse2009. p. 22 p.
32. Murphy AM, Grohmann GS, Christopher PJ, Lopez WA, Davey GR, Millsom RH. An Australia-wide outbreak of gastroenteritis from oysters caused by Norwalk virus. The Medical journal of Australia. 1979;2(7):329-33.
33. Morse DL, Guzewich JJ, Hanrahan JP, Stricof R, Shayegani M, Deibel R, et al. Widespread Outbreaks of Clam- and Oyster-Associated Gastroenteritis. N Engl J Med. 1986;314(11):678-81.
34. Le Guyader FS, Le Saux J-C, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, et al. Aichi Virus, Norovirus, Astrovirus, Enterovirus, and Rotavirus Involved in Clinical Cases from a French Oyster-Related Gastroenteritis Outbreak. J Clin Microbiol. 2008;46(12):4011-7.
35. Le Guyader FS, Bon F, DeMedici D, Parnaudeau S, Bertone A, Crudeli S, et al. Detection of multiple noroviruses associated with an international

gastroenteritis outbreak linked to oyster consumption. J Clin Microbiol. 2006;44(11):3878-82.
36. Riou P, Le Saux JC, Dumas F, Caprais MP, Le Guyader SF, Pommepuy M. Microbial impact of small tributaries on water and shellfish quality in shallow coastal areas. Water Research. 2007;41(12):2774-86.
37. Derx J, Schijven J, Sommer R, Zoufal-Hruza CM, van Driezum IH, Reischer G, et al. QMRAcatch: Human-Associated Fecal Pollution and Infection Risk Modeling for a River/Floodplain Environment. J Environ Qual. 2016;45(4):1205-14.
38. Diston D, Sinreich M, Zimmermann S, Baumgartner A, Felleisen R. Evaluation of molecular- and culture-dependent MST markers to detect fecal contamination and indicate viral presence in good quality groundwater. Environ Sci Technol. 2015;49(12):7142-51.
39. Harrault L, Jardé E, Jeanneau L, Petitjean P. Development of the analysis of fecal stanols in the oyster Crassostrea gigas and identification of fecal contamination in shellfish harvesting areas. Lipids. 2014;49(6):597-607.
40. Geary PM, Davies CM. Bacterial source tracking and shellfish contamination in a coastal catchment. Water Science and Technology. 2003;47(7-8):95-100.
41. Crowther J, Kay D, Campos CJ, Morgan OC. Sanitary profiles of selected shellfish water catchments pre- and post-improvements in sewerage infrastructure. Cefas; 2011.
42. Ventrone I, Schaeffer J, Ollivier J, Parnaudeau S, Pepe T, Le Pendu J, et al. Chronic or Accidental Exposure of Oysters to Norovirus: Is There Any Difference in Contamination? J Food Prot. 2013;76(3):505-9.
43. Montazeri N, Maite M, Liu D, Cormier J, Landry M, Shackleford J, et al. Surveillance of Enteric Viruses and Microbial Indicators in the Eastern Oysters (Crassostrea virginica) and Harvest Waters along Louisiana Gulf Coast. J Food Sci. 2015;80(5):M1075-M82.
44. Onsite sewage systems: BC Ministroy of Environment; [Available from: http://www.env.gov.bc.ca/wat/wq/nps/NPS_Pollution/Onsite_Sewage_Systems2/Onsite_Main.htm.
45. United States Environmental Protection Agency. Primer for municipal wastewater treatment systems. In: Office of Water and Wastewater Management, editor. Washington, DC2004. p. 30.

46. Public health act sewerage system regulation, (2004).
47. Borchardt MA, Chyou P-H, DeVries EO, Belongia EA. Septic system density and infectious diarrhea in a defined population of children. Environ Health Perspect. 2003;111(5):742-8.
48. Yates MV. Septic Tank Density and Ground-Water Contamination. Ground Water. 1985;23(5):586-91.
49. Wallender EK, Ailes EC, Yoder JS, Roberts VA, Brunkard JM. Contributing Factors to Disease Outbreaks Associated with Untreated Groundwater. Groundwater. 2014;52(6):886-97.
50. Hynds PD, Thomas MK, Pintar KDM. Contamination of Groundwater Systems in the US and Canada by Enteric Pathogens, 1990–2013: A Review and Pooled-Analysis. PLoS One. 2014;9(5):e93301.
51. Yates MV, Yates SR, Warrick AW, Gerba CP. Use of geostatistics to predict virus decay rates for determination of septic tank setback distances. Appl Environ Microbiol. 1986;52(3):479-83.
52. Viau EJ, Lee D, Boehm AB. Swimmer Risk of Gastrointestinal Illness from Exposure to Tropical Coastal Waters Impacted by Terrestrial Dry-Weather Runoff. Environ Sci Technol. 2011;45(17):7158-65.
53. Symonds EM, Sinigalliano C, Gidley M, Ahmed W, McQuaig-Ulrich SM, Breitbart M. Faecal pollution along the southeastern coast of Florida and insight into the use of pepper mild mottle virus as an indicator. J Appl Microbiol. 2016;121(5):1469-81.
54. Lipp EK, Farrah SA, Rose JB. Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. Mar Pollut Bull. 2001;42(4):286-93.
55. Paul JH, McLaughlin MR, Griffin DW, Lipp EK, Stokes R, Rose JB. Rapid movement of wastewater from on-site disposal systems into surface waters in the Lower Florida Keys. Estuaries. 2000;23(5):662-8.
56. Paul JH, Rose JB, Brown J, Shinn EA, Miller S, Farrah SR. Viral Tracer Studies Indicate Contamination of Marine Waters by Sewage Disposal Practices in Key Largo, Florida. Appl Environ Microbiol. 1995;61(6):2230-4.
57. A C, J L, M P-H, R L. Spatial and temporal pattern of norovirus contamination in a Pacific oyster fishery. Proceedings of the 7th International Conference on Molluscan Shellfish Safety; 14 to 19 June 2009; Nantes, France2009. p. 81-7.

58. Borchardt MA, Bradbury KR, Alexander EC, Kolberg RJ, Alexander SC, Archer JR, et al. Norovirus Outbreak Caused by a New Septic System in a Dolomite Aquifer. Ground Water. 2011;49(1):85-97.
59. Gunnarsdottir MJ, Gardarsson SM, Andradottir HO. Microbial contamination in groundwater supply in a cold climate and coarse soil: case study of norovirus outbreak at Lake Mývatn, Iceland. Hydrology Research. 2013;44(6):1114-28.
60. Craun G. Health aspects of groundwater pollution. In: Bitton G, Gerba CP, editors. Groundwater pollution microbiology: John Wiley & Sons; 1984.
61. Li Y, Guo HX, Xu ZH, Zhou XT, Zhang HL, Zhang LJ, et al. An outbreak of norovirus gastroenteritis associated with a secondary water supply system in a factory in south China. BMC Public Health. 2013;13.
62. Desenclos JC, Klontz KC, Wilder MH, Nainan OV, Margolis HS, Gunn RA. A multistate outbreak of hepatitis A caused by the consumption of raw oysters. Am J Public Health. 1991;81(10):1268-72.
63. Report to Congress: Impacts and Control of CSOs and SSOs. In: Agency USEP, editor. Washington DC2004.
64. Control TGVSaDDEMaQ. Wastewater Annual Report 2014. Metro Vancouver, Department LWS; 2014.
65. Rodríguez RA, Gundy PM, Rijal GK, Gerba CP. The Impact of Combined Sewage Overflows on the Viral Contamination of Receiving Waters. Food Environ Virol. 2012;4(1):34-40.
66. Campos CJ, Avant J, Lowther J, Till D, Lees D. Levels of norovirus and E. coli in untreated, biologically treated and UV-disinfected sewage effluent discharged to a shellfish water. Journal of Water Resource and Protection. 2013;2013.
67. McCallum D, Macdonald R, Ham P. Evolution of Urban Stormwater Environmental Assessment Methods: A Case Study of the Greater Vancouver Region, British Columbia. Proceedings of the Water Environment Federation. 2000(6):2517-34.
68. Combined Sewer Overflow Technology Fact Sheet Retention Basins. In: Agency USEP, editor. Washington DC: Office of Water; 1999.
69. Baker-Austin C, Campos CJ, Turner A, Higman W, Lees D. Impacts of climate change on human health. MCCIP Science Review 2013. 2013:257-62.

70. Riou P, Le Saux J-C, Dumas F, Le Guyader S, Le Goff R, Maheux F, et al. The role of models in assessing the impact of sewage overflows on feacal water contamination. J Shellfish Res. 2008.
71. Sterk A, de Man H, Schijven JF, de Nijs T, de Roda Husman AM. Climate change impact on infection risks during bathing downstream of sewage emissions from CSOs or WWTPs. Water Research. 2016;105:11-21.
72. Le Mennec C, Parnaudeau S, Rumebe M, Le Saux JC, Piquet JC, Le Guyader SF. Follow-Up of Norovirus Contamination in an Oyster Production Area Linked to Repeated Outbreaks. Food Environ Virol. 2017;9(1):54-61.
73. Guillois-Becel Y, Couturier E, Le Saux JC, Roque-Afonso AM, Le Guyader FS, Le Goas A, et al. An Oyster-Associated Hepatitis A Outbreak In France In 2007. Eurosurveillance. 2009;14(10).
74. Nygard K, Torven M, Ancker C, Knauth SB, Hedlund KO, Giesecke J, et al. Emerging genotype (GGIIb) of norovirus in drinking water, Sweden. Emerg Infect Dis. 2003;9(12):1548-52.
75. Fitzgerald TL, Merritt TD, Zammit A, McLeod C, Landinez LM, White PA, et al. An outbreak of norovirus genogroup II associated with New South Wales oysters. Communicable Diseases Intelligence Quarterly Report. 2014;38(1):E9-E15.
76. Flannery J, Keaveney S, Rajko-Nenow P, O'Flaherty V, Dore W. Norovirus and FRNA bacteriophage determined by RT-qPCR and infectious FRNA bacteriophage in wastewater and oysters. Water Research. 2013;47(14):5222-31.
77. Passerat J, Ouattara NK, Mouchel JM, Rocher V, Servais P. Impact of an intense combined sewer overflow event on the microbiological water quality of the Seine River. Water Research. 2011;45(2):893-903.
78. Jagai JS, Li Q, Wang S, Messier KP, Wade TJ. Extreme Precipitation and Emergency Room Visits for Gastrointestinal Illness in Areas with and without Combined Sewer Systems: An Analysis of Massachusetts Data, 2003-2007. Environ Health Perspect. 2015;123(9):873-9.
79. Katayama H, Okuma K, Furumai H, Ohgaki S. Series of surveys for enteric viruses and indicator organisms in Tokyo Bay after an event of combined sewer overflow. Water Science and Technology. 2004;50(1):259-62.

80. Kay D, Kershaw S, Lee R, Wyer MD. Impact of intermittent discharges on the microbiological quality of shellfish. In: Agency DE, editor.: UK Water Industry Research Limited; 2007.
81. Grodzki M, Ollivier J, Le Saux JC, Piquet JC, Noyer M, Le Guyader FS. Impact of Xynthia Tempest on Viral Contamination of Shellfish. Appl Environ Microbiol. 2012;78(9):3508-11.
82. Brake F, Kiermeier A, Ross T, Holds G, Landinez L, McLeod C. Spatial and Temporal Distribution of Norovirus and E. coli in Sydney Rock Oysters Following a Sewage Overflow into an Estuary. Food Environ Virol. 2017.
83. Devane ML, Moriarty EM, Wood D, Webster-Brown J, Gilpin BJ. The impact of major earthquakes and subsequent sewage discharges on the microbial quality of water and sediments in an urban river. Sci Total Environ. 2014;485:666-80.
84. The McIlvaine Company. Municipal Wastewater 2017 [Available from: http://www.mcilvainecompany.com/generic%20applications/water/mncpl%20wastewater.htm.
85. MetroVancouver. How Wasterwater is Treated 2017 [Available from: http://www.metrovancouver.org/services/liquid-waste/treatment/treatment-plants/how-wastewater-treated/Pages/default.aspx.
86. Francy DS, Stelzer EA, Bushon RN, Brady AM, Williston AG, Riddell KR, et al. Comparative effectiveness of membrane bioreactors, conventional secondary treatment, and chlorine and UV disinfection to remove microorganisms from municipal wastewaters. Water Res. 2012;46(13):4164-78.
87. Government of Canada. Municipal effluent chlorination and dechlorination: principles, technologies and practices 2013 [Available from: https://www.canada.ca/en/environment-climate-change/services/wastewater/resource-documents/municipal-effluent-chlorination-principles-technologies-practices.html.
88. Widipedia. Sewage treatment 2017 [Available from: https://en.wikipedia.org/wiki/Sewage_treatment.
89. Wastewater Systems Effluent Regulations, (2012).
90. Powell R, Greater Nanaimo Pollution Control Centre. Coliform and viral testing (inquiry by phone). In: Received by L. McIntyre, editor. 2017.
91. Environment Canada. Municipal Wastewater Treatment Indicator 2017 [Available from: https://www.ec.gc.ca/indicateurs-indicators/default.asp?lang=En&n=2647AF7D-1.

92. Environment Canada. 2011 Municipal Water Use Report. Municipal water use 2009 statistics.: Government of Canada; 2011. Report No.: En11-2/2009E-PDF
93. Government of Canada. Canada Gazette: archived Wastewater Systems Effluent Regulations. Regulatory impact analysis statement (not part of the Regulations). 2012 [Available from: http://www.gazette.gc.ca/rp-pr/p2/2012/2012-07-18/html/sor-dors139-eng.html.
94. Eftim SE, Hong T, Soller J, Boehm A, Warren I, Ichida A, et al. Occurrence of norovirus in raw sewage – A systematic literature review and meta-analysis. Water Research. 2017;111:366-74.
95. Pouillot R, Van Doren JM, Woods J, Plante D, Smith M, Goblick G, et al. Meta-Analysis of the Reduction of Norovirus and Male-Specific Coliphage Concentrations in Wastewater Treatment Plants. Appl Environ Microbiol. 2015;81(14):4669-81.
96. Campos CJ, Lees DN. Environmental transmission of human noroviruses in shellfish waters. Appl Environ Microbiol. 2014;80(12):3552-61.
97. Dienus O, Sokolova E, Nyström F, Matussek A, Löfgren S, Blom L, et al. Norovirus Dynamics in Wastewater Discharges and in the Recipient Drinking Water Source: Long-Term Monitoring and Hydrodynamic Modeling. Environ Sci Technol. 2016;50(20):10851-8.
98. Campos CJA, Avant J, Lowther J, Till D, Lees DN. Human norovirus in untreated sewage and effluents from primary, secondary and tertiary treatment processes. Water Research. 2016;103:224-32.
99. Hewitt J, Leonard M, Greening GE, Lewis GD. Influence of wastewater treatment process and the population size on human virus profiles in wastewater. Water Res. 2011;45(18):6267-76.
100. Flannery J, Keaveney S, Rajko-Nenow P, O'Flaherty V, Doré W. Concentration of Norovirus during Wastewater Treatment and Its Impact on Oyster Contamination. Appl Environ Microbiol. 2012;78(9):3400-6.
101. Montazeri N, Goettert D, Achberger EC, Johnson CN, Prinyawiwatkul W, Janes ME. Pathogenic Enteric Viruses and Microbial Indicators during Secondary Treatment of Municipal Wastewater. Appl Environ Microbiol. 2015;81(18):6436-45.
102. Kay D, Kershaw S, Lee R, Wyer MD, Watkins J, Francis C. Results of field investigations into the impact of intermittent sewage discharges on

the microbiological quality of wild mussels (Mytilus edulis) in a tidal estuary. Water Research. 2008;42(12):3033-46.
103. Campos CJ, Avant A, Lowther J, Till D, Lees D. Levels of Norovirus and E. coli in Untreated, Biologically Treated and UV-Disinfected Sewage Effluent Discharged to a Shellfish Water. J Water Res Prot 2013;5:978-82.
104. Qiu Y, Lee BE, Neumann N, Ashbolt N, Craik S. Assessment of human virus removal during municipal wastewater treatment in Edmonton, Canada. J Appl Microbiol. 2015;119(6):1729-39.
105. Nordgren J, Matussek A, Mattsson A, Svensson L, Lindgren PE. Prevalence of norovirus and factors influencing virus concentrations during one year in a full-scale wastewater treatment plant. Water Res. 2009;43(4):1117-25.
106. Suzuki Y, Narimatsu S, Furukawa T, Mekata T, Kono T, Sakai M, et al. Removal of Noroviruses from Municipal Wastewater by Foam Separation using Dispersed Air-Bubbles and Surface-Active Substance. Separation Science and Technology. 2009;44(3):569-84.
107. Barrett M, Fitzhenry K, O'Flaherty V, Dore W, Keaveney S, Cormican M, et al. Detection, fate and inactivation of pathogenic norovirus employing settlement and UV treatment in wastewater treatment facilities. Sci Total Environ. 2016;568:1026-36.
108. Winterbourn JB, Clements K, Lowther JA, Malham SK, McDonald JE, Jones DL. Use of Mytilus edulis biosentinels to investigate spatial patterns of norovirus and faecal indicator organism contamination around coastal sewage discharges. Water Research. 2016;105:241-50.
109. Goblick GN, Anbarchian JM, Woods J, Burkhardt W, Calci K. Evaluating The Dilution Of Wastewater Treatment Plant Effluent And Viral Impacts On Shellfish Growing Areas In Mobile Bay, Alabama. J Shellfish Res. 2011;30(3):979-87.
110. Hassard F, Sharp JH, Taft H, LeVay L, Harris JP, McDonald JE, et al. Critical Review on the Public Health Impact of Norovirus Contamination in Shellfish and the Environment: A UK Perspective. Food Environ Virol. 2017.
111. Minden Gardnerville Sanitation District. Helpful Information, Glossary of Wastewater Terms 2017 [Available from: http://www.mgsdistrict.org/helpful-information/educational/.
112. Appleton H, Pereira MS. A possible virus aetiology in outbreaks of food-poisoning from cockles. Lancet. 1977;1(8015):780-1.

113. Yu Y, Cai H, Hu L, Lei R, Pan Y, Yan S, et al. Molecular epidemiology of oyster-related human noroviruses and their global genetic diversity and temporal-geographical distribution from 1983 to 2014. Appl Environ Microbiol. 2015;81(21):7615-24.
114. Gill ON, Cubitt WD, McSwiggan DA, Watney BM, Bartlett CL. Epidemic of gastroenteritis caused by oysters contaminated with small round structured viruses. Br Med J (Clin Res Ed). 1983;287(6404):1532-4.
115. Symes SJ, Gunesekere IC, Marshall JA, Wright PJ. Norovirus mixed infection in an oyster-associated outbreak: an opportunity for recombination. Arch Virol. 2007;152(6):1075-86.
116. Sugieda M, Nakajima K, Nakajima S. Outbreaks of Norwalk-like virus-associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. Epidemiol Infect. 1996;116(3):339-46.
117. Halliday ML, Kang LY, Zhou TK, Hu MD, Pan QC, Fu TY, et al. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. J Infect Dis. 1991;164(5):852-9.
118. Prato R, Martinelli D, Tafuri S, Barbuti G, Quarto M, Germinario CA, et al. Safety of shellfish and epidemiological pattern of enterically transmitted diseases in Italy. Int J Food Microbiol. 2013;162(2):125-8.
119. Berg DE, Kohn MA, Farley TA, McFarland LM. Multi-state outbreaks of acute gastroenteritis traced to fecal-contaminated oysters harvested in Louisiana. J Infect Dis. 2000;181 Suppl 2:S381-6.
120. Kohn MA, Farley TA, Ando T, Curtis M, Wilson SA, Jin Q, et al. An outbreak of norwalk virus gastroenteritis associated with eating raw oysters - implications for maintaining safe oyster beds. JAMA. 1995;273(6):466-71.
121. Control CfD, Prevention. Viral gastroenteritis associated with consumption of raw oysters--Florida, 1993. MMWR Morbidity and mortality weekly report. 1994;43(24):446.
122. Centers for Disease C, Prevention. Multistate outbreak of viral gastroenteritis associated with consumption of oysters--Apalachicola Bay, Florida, December 1994-January 1995. MMWR Morb Mortal Wkly Rep. 1995;44(2):37-9.
123. Christensen B, Lees D, Wood K, Bjergskov T, Green J. Human enteric viruses in oysters causing a large outbreak of human food borne infection in 1996/97. J Shellfish Res. 1998;17(5):1633-5.

124. Lopalco PL, Malfait P, Salmaso S, Germinario C, Quarto M, Barbuti S, et al. A persisting outbreak of hepatitis A in Puglia, Italy, 1996: epidemiological follow-up. Euro Surveill. 1997;2(4):31-2.
125. Iritani N, Kaida A, Kubo H, Abe N, Goto K, Ogura H, et al. Molecular epidemiology of noroviruses detected in seasonal outbreaks of acute nonbacterial gastroenteritis in Osaka City, Japan, from 1996-1997 to 2008-2009. J Med Virol. 2010;82(12):2097-105.
126. Shieh Y, Monroe SS, Fankhauser RL, Langlois GW, Burkhardt W, 3rd, Baric RS. Detection of norwalk-like virus in shellfish implicated in illness. J Infect Dis. 2000;181 Suppl 2:S360-6.
127. Sanchez G, Pinto RM, Vanaclocha H, Bosch A. Molecular characterization of hepatitis a virus isolates from a transcontinental shellfish-borne outbreak. J Clin Microbiol. 2002;40(11):4148-55.
128. Simmons G, Greening G, Gao WZ, Campbell D. Raw oyster consumption and outbreaks of viral gastroenteritis in New Zealand: evidence for risk to the public's health. Aust N Z J Public Health. 2001;25(3):234-40.
129. Le Guyader FS, Neill FH, Dubois E, Bon F, Loisy F, Kohli E, et al. A semiquantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak. Int J Food Microbiol. 2003;87(1-2):107-12.
130. Cheng PK, Wong DK, Chung TW, Lim WW. Norovirus contamination found in oysters worldwide. J Med Virol. 2005;76(4):593-7.
131. Noda M, Fukuda S, Nishio O. Statistical analysis of attack rate in norovirus foodborne outbreaks. Int J Food Microbiol. 2008;122(1-2):216-20.
132. Iritani N, Kaida A, Abe N, Kubo H, Sekiguchi J-I, Yamamoto SP, et al. Detection and genetic characterization of human enteric viruses in oyster-associated gastroenteritis outbreaks between 2001 and 2012 in Osaka City, Japan. J Med Virol. 2014;86(12):2019-25.
133. Nakagawa-Okamoto R, Arita-Nishida T, Toda S, Kato H, Iwata H, Akiyama M, et al. Detection of multiple sapovirus genotypes and genogroups in oyster-associated outbreaks. Jpn J Infect Dis. 2009;62(1):63-6.
134. Prato R, Lopalco PL, Chironna M, Barbuti G, Germinario C, Quarto M. Norovirus gastroenteritis general outbreak associated with raw shellfish consumption in South Italy. BMC Infect Dis. 2004;4.

135. Webby RJ, Carville KS, Kirk MD, Greening G, Ratcliff RM, Crerar SK, et al. Internationally Distributed Frozen Oyster Meat Causing Multiple Outbreaks of Norovirus Infection in Australia. Clin Infect Dis. 2007;44(8):1026-31.
136. Ng TL, Chan PP, Phua TH, Loh JP, Yip R, Wong C, et al. Oyster-associated outbreaks of Norovirus gastroenteritis in Singapore. J Infect. 2005;51(5):413-8.
137. David ST, McIntyre L, MacDougall L, Kelly D, Liem S, Schallie K, et al. An outbreak of norovirus caused by consumption of oysters from geographically dispersed harvest sites, British Columbia, Canada, 2004. Foodborne Pathog Dis. 2007;4(3):349-58.
138. Pontrelli G, Boccia D, M DIR, Massari M, Giugliano F, Celentano LP, et al. Epidemiological and virological characterization of a large community-wide outbreak of hepatitis A in southern Italy. Epidemiol Infect. 2008;136(8):1027-34.
139. Shieh YC, Khudyakov YE, Xia G, Ganova-Raeva LM, Khambaty FM, Woods JW, et al. Molecular Confirmation of Oysters as the Vector for Hepatitis A in a 2005 Multistate Outbreak. J Food Prot. 2007;70(1):145-50.
140. Nenonen NP, Hannoun C, Olsson MB, Bergstrom T. Molecular analysis of an oyster-related norovirus outbreak. J Clin Virol. 2009;45(2):105-8.
141. Lowther JA, Gustar NE, Hartnell RE, Lees DN. Comparison of Norovirus RNA Levels in Outbreak-Related Oysters with Background Environmental Levels. Journal of Food Protection®. 2012;75(2):389-93.
142. Le Guyader FS, Krol J, Ambert-Balay K, Ruvoen-Clouet N, Desaubliaux B, Parnaudeau S, et al. Comprehensive analysis of a norovirus-associated gastroenteritis outbreak, from the environment to the consumer. J Clin Microbiol. 2010;48(3):915-20.
143. Iizuka S, Oka T, Tabara K, Omura T, Katayama K, Takeda N, et al. Detection of Sapoviruses and Noroviruses in an Outbreak of Gastroenteritis Linked Genetically to Shellfish. J Med Virol. 2010;82(7):1247-54.
144. Smith A, McCarthy N, Saldana L, Ihekweazu C, McPhedran K, Adak G, et al. A large foodborne outbreak of norovirus in diners at a restaurant in England between January and February 2009. Epidemiol Infect. 2012;140(09):1695-701.

145. Alfano-Sobsey E, Sweat D, Hall A, Breedlove F, Rodriguez R, Greene S, et al. Norovirus outbreak associated with undercooked oysters and secondary household transmission. Epidemiol Infect. 2012;140(2):276-82.
146. McLaughlin J. Norovirus Outbreak due to Consumption of Raw Oysters—Alaska, 2009.
147. Rajko-Nenow P, Keaveney S, Flannery J, McIntyre A, Dore W. Norovirus genotypes implicated in two oyster-related illness outbreaks in Ireland. Epidemiol Infect. 2014;142(10):2096-104.
148. Westrell T, Dusch V, Ethelberg S, Harris J, Hjertqvist M, Jourdan-da Silva N, et al. Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. Euro Surveill. 2010;15(12).
149. McIntyre L, Galanis E, Mattison K, Mykytczuk O, Buenaventura E, Wong J, et al. Multiple clusters of norovirus among shellfish consumers linked to symptomatic oyster harvesters. J Food Prot. 2012;75(9):1715-20.
150. Boxman IL, Verhoef L, Vennema H, Ngui S, Friesema IH, Whiteside C, et al. International linkage of two food-borne hepatitis A clusters through traceback of mussels, the Netherlands, 2012. Eurosurveillance. 2016;21(3):2-10.
151. Loury P, le Guyader FS, le Saux JC, Ambert-Balay K, Parrot P, Hubert B. A norovirus oyster-related outbreak in a nursing home in France, January 2012. Epidemiol Infect. 2015;143(12):2486-93.
152. Lodo KL, Veitch MGK, Green ML. An outbreak of norovirus linked to oysters in Tasmania. Communicable Diseases Intelligence Quarterly Report. 2014;38(1):E16-E9.
153. Cho H, Lee S, Lee M, Hur E, Lee J, Park P, et al. An outbreak of norovirus infection associated with fermented oyster consumption in South Korea, 2013. Epidemiol Infect. 2016:1-6.
154. Dam Rasmussen L, Schultz AC, Uhrbrand K, Jensen T, Kølsen Fischer T, Rasmussen LD, et al. Molecular Evidence of Oysters as Vehicle of Norovirus GII.P17-GII.17. Emerg Infect Dis. 2016;22(11):2024-5.
155. Public Health Agency of Canada. Public Health Notice – Ongoing outbreak of norovirus and gastrointestinal illnesses linked to raw and undercooked oysters from British Columbia 2017 [Available from: http://www.phac-aspc.gc.ca/phn-asp/2017/outbreak-norovirus-eclosion-eng.php.