Risk assessment to support development of advice and guidance to manage outbreaks of norovirus in oysters

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Executive Summary

This report has been commissioned to assess the public health risk associated with consumption of raw oysters with a range of norovirus RNA levels.

Norovirus infection is typically a mild, self-limiting illness, and foodborne transmission is an important route, responsible for an estimated 16% of cases of norovirus. Between 2017 and 2023 in Scotland, and between 2013 – 2022 in England, there were, in total, 1,566 reported cases of norovirus infection linked to oysters, of which 4 were hospitalised. Data is not routinely collected from Wales and was not available from Northern Ireland. Cases are more frequent during winter months.

Detection of viral particles uses a polymerase chain reaction (PCR) method but this cannot distinguish between infectious and non-infectious norovirus. Cefas in England and the Marine Institute in the Republic of Ireland are the only laboratories accredited for PCR quantification of norovirus in oysters in the British Isles. Other laboratories may provide norovirus quantification, using methods comparable to the International Standard method ISO 15216. There can be variability due to differences between laboratories and the starting material, with the latter especially affecting the extraction efficiency.

Estimates of the norovirus dose required to make 50% of susceptible individuals ill vary between studies from a thousand to hundreds of thousands of particles, with modelling studies suggesting it may be even lower. However, there is a broad consensus that even low doses of infectious particles can be sufficient to make susceptible individuals ill.

Norovirus contaminates oysters due to human sewage discharges around oyster beds. Oysters bio-accumulate the virus in their digestive glands, with levels of norovirus significantly higher in winter months than in summer months. As part of this assessment, we compared norovirus levels in oysters sampled at retail to levels in oyster batches linked to outbreaks. Outbreak batches had significantly higher levels, with geometric mean of 874 norovirus genome copies/g compared to 24 genome copies/g in retail batches, although results varied over a broad range for both groups.

There is uncertainty regarding the frequency with which oysters are consumed in the UK, but we estimate that around 4 million oyster meals are consumed each year, with an average portion size of six oysters, which weigh 30 g per oyster on average.

We conclude that the risk of illness is **unknown** in the absence of risk factors such as sewerage spills, epidemiological linkage to outbreaks, high *E. coli* levels or high rainfall. Should oysters be consumed without further treatment and in conjunction with scenarios such as potential human wastewater contamination from sewage spills or if the batches are linked to outbreaks, then at 1-200 norovirus genome copies/g the risk is **low - medium*** (rare, but does occur - occurs regularly) *(*the likelihood of illness when consuming other oysters from that bed is **low-medium** in instances of test results below 200 norovirus genome copies per gram. This is to recognise that the likelihood may be assessed **low** in outbreaks with a small number of reported illnesses (or on the basis of other risk factors), or could be assessed **medium** in outbreaks with a large number of reported illnesses (or on the basis of other risk factors)).* At 201-500 copies/g the risk is **medium** (occurs regularly), at 501-1,000 copies/g the risk is **high** (occurs very often) and >1,000 copies/g the risk is **very high** (events occur almost certainly) with all scenarios having **high** uncertainty.

Lay Summary

This report has been written to assess the public health risk associated with consumption of raw oysters with a range of norovirus test results. In England, between 2013 – 2022, there were 1,307 reported cases of norovirus infection linked to oysters. In Scotland, between 2017 – 2023, there were 259 reported cases of norovirus linked to oysters. Data is not routinely collected from Wales and was not available from Northern Ireland. Norovirus is a type of virus which can cause a mild illness. Norovirus can sometimes contaminate food, accounting for up to 16% of infections. Oysters are a notable source of norovirus, in terms of risk per serving, particularly because oysters are typically consumed raw.

The virus can be detected and quantified in foods including oysters, but tests can't distinguish between infectious virus and damaged virus which is unable to cause infection. Cefas in England and the Marine Institute in the Republic of Ireland are the only labs in the British Isles which are certified to carry out the test in oysters to a recognised quality standard, known as ISO 15216. Other labs might be able to carry out similar tests, but they are not certified to test using ISO 15216. In addition, even two laboratories certified to ISO 15216, testing oysters from the same batch, might find different results because of variability between individual oysters and laboratory-specific differences.

The number of infectious norovirus particles required to make 50% of susceptible people ill varies between studies, from a thousand to hundreds of thousands of viruses, with some modelling studies suggesting it may be even lower. Even low amounts of virus can be sufficient to make people ill.

Norovirus contamination in oysters largely occurs due to human sewage releases close to oyster beds. Oysters are filter feeders who take up norovirus as they filter seawater. The levels of norovirus vary widely depending on season, with higher levels in winter months than in summer months. We compared norovirus levels in oysters at retail to levels in oyster batches linked to outbreaks and found that outbreak batches had significantly higher levels.

There is uncertainty about the levels of oyster consumption in the UK. But we estimate that around 4 million oyster meals are consumed each year, with an average portion size of 6 oysters, which weigh 30 g per oyster on average.

We conclude that if oysters are eaten raw and there is potential human wastewater contamination from sewerage spills or if the oyster batch is linked to outbreaks, there is a risk of illness from norovirus. If the norovirus in the oysters is 1-200 copies/g, the risk is **low - medium*** (rare, but does occur - occurs regularly) (*the likelihood will depend on the specific circumstances of the incident and the additional information available), at 201-500 copies/g the risk is **medium** (occurs regularly), at 501-1,000 copies/g the risk is **high** (occurs very often) and >1,000 copies/g the risk is **very high** (events occur almost certainly). All these scenarios have **high** uncertainty. The risk of illness is **unknown** if only the norovirus levels are available, without further information.

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Abbreviations

Term	Definition
FBO	Food business operator
FSA	Food Standards Agency
FSS	Food Standards Scotland
LOD	Limit of detection
LOQ	Limit of quantification
PCR	Polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
UKHSA	United Kingdom Health Security Agency

1. Statement of Purpose

1.1 Background

This assessment has been commissioned in response to recurring outbreaks of norovirus linked to the consumption of raw oysters. Each outbreak requires food safety and health protection resource to manage, as well as having a direct and indirect impact on consumers and on businesses involved.

There has been a desire by Local Authorities (LA), Food Business Operators (FBOs) and UK Health Security Agency (UKHSA) for guidance when dealing with norovirus outbreaks. There are no norovirus criteria defined in legislation, nor any published guidance in the UK which relates the levels of norovirus in food, in particular oysters, to the level of risk to consumers of the raw product.

Managing the risks from oysters during norovirus outbreaks is difficult – closing a shellfish bed and ceasing harvesting for a long time is economically damaging to the business. On the other hand, reopening too quickly can lead to further norovirus cases. As FBOs in the UK have a responsibility under Article 14 of Retained EU Regulation (EC) No. 178/2002 (GB) and EU Regulation (EC) No. 178/2002 (NI), to not place unsafe food on the market, some FBOs take a business decision to cease harvesting for an extended period of time.

There are technical difficulties in using the results of polymerase chain reaction (PCR) methods to assess the norovirus risk to public health as PCR cannot assess infectivity, and because of this, such methods have not been a key aspect of FSA and FSS risk management advice in recent years. In addition, due to interlaboratory differences, conflicting results obtained from different laboratories can complicate the ability to issue advice. There is a need for improved risk management options, particularly with respect to outbreaks, and for re-opening of shellfish beds that have been contaminated with norovirus. This report assesses scientific literature, laboratory sampling data and outbreak data to establish a relationship between measured levels of norovirus RNA in oysters and their association with cases of illness, and describe the causes of interlaboratory differences, with the aim of informing risk management advice.

1.2 Legislation

There is no regulatory limit for norovirus in Section VII of Retained EU Regulation (EC) No. 853/2004 (GB) and Regulation (EC) No 853/2004 (NI), or Retained EU Regulation (EC) No. 2073/2005 (GB) and EU Regulation (EC) No. 2073/2005 (NI) relating to any food commodity, including live bivalve molluscs, although there are specific microbiological criteria for other enteric foodborne pathogens and indicators including *Salmonella* spp. and *Escherichia coli* (*E. coli*). Shellfish producers are however, still required to meet their general obligations in food law, e.g. Article 14 of Retained EU Regulation (EC) No. 178/2002 (GB) and EU Regulation (EC) No. 178/2002 (NI) which prohibits placing food on the market if it is unsafe.

1.3 Scope of risk assessment

What is the public health risk associated with consumption of raw oysters with a range of norovirus RNA levels, including 200 and 500 genome copies/g?

What is the capacity of both official laboratories and commercial laboratories in GB, Northern Ireland and the Republic of Ireland to carry out quantitative real time PCR for norovirus to relevant levels?

How do ISO 15216 (or equivalent) method results below the limit of detection or quantification (LOD/LOQ) and inter-laboratory differences in LOD/LOQ affect the interpretation of results and the risk level?

1.4 Out of Scope

- The effects of post-harvest treatments such as conditioning, relaying and depuration in natural or man-made environments on norovirus levels
- Whether intact norovirus particles can be differentiated from other norovirus genetic material to indicate potential infectivity
- Longevity of norovirus infectivity in oysters
- Links between *E. coli* levels and norovirus outbreaks
- Exclusion/Buffer zones (zones put in place around shellfish production areas to provide safe separation from wastewater discharges)

- Cooked oysters (70 °C for 2 minutes or equivalent)
- Live bivalve molluscs other than oysters

2. Hazard identification

Noroviruses are a genetically diverse group of single-stranded positive-sense RNA, non-enveloped viruses belonging to the family *Caliciviridae*. Noroviruses can be genetically classified into at least ten different genogroups (GI-GX), which can be further divided into different genetic clusters or genotypes. Genogroups I, II and IV infect humans (Chhabra *et al.*, 2019). Most noroviruses that infect humans belong to genogroups GI and GII.

Noroviruses are transmitted by the faecal-oral route, predominantly person-to-person and through airborne particles, but also through contamination of food, water, and fomites. Noroviruses usually cause outbreaks of illness in closed settings (hospitals, residential homes and schools) where close contact between asymptomatic and symptomatic individuals results in transmission of the virus (de Graaf, van Beek and Koopmans, 2016). Symptoms are mild and self-limiting. Noroviruses are the most common cause of acute foodborne gastroenteritis in humans of all age groups in the UK (Holland and Mahmoudzadeh, 2020). Although cases tend to be through person-person contact, foodborne transmission of norovirus is high. An FSA-funded quantitative microbiological risk assessment estimated the proportion of foodborne norovirus cases in the UK at 16%, with significant uncertainty[1] (O'Brien *et al.*, 2019); an FSA technical review of this model suggested the median estimate could be as low as 12%, but that uncertainty remained very high (Gherman *et al.*, 2019), and an individual-based modelling approach based on some of the same sources (O'Brien *et al.*, 2019) estimated this as high as 35%. Poor hygiene can also lead to the spread of infection from infected food handlers (Atmar and Estes, 2006). Noroviruses cannot grow in food and they can be inactivated by cooking,

[1] As defined by EFSA: 'Uncertainty is used as a general term referring to all types of limitations in available knowledge that affect the range and probability of possible answers to an assessment question'

but are quite resistant to inactivation by cold temperatures, low pH and hand sanitisers (Nims and Plavsic, 2013; Barclay *et al.*, 2014)

The probability of becoming infected increases with the dose of norovirus ingested but depends also on the characteristics of the strain and the host (Teunis *et al.*, 2020). There is evidence to suggest that very low levels can cause infection (Teunis *et al.*, 2008; Lopman *et al.*, 2012; Atmar *et al.*, 2014). Detection of norovirus is commonly through detection of the viral RNA by reverse transcription polymerase chain reaction (RT-PCR). PCR detects nucleic acid and is not able to differentiate between infectious and non-infectious virus; RNA levels are an indication that a viral risk may exist, rather than a direct proof of risk. No standardised cell-based assay allowing the direct measurement of infectivity exists for human norovirus. To further complicate matters, the relationship between the number of infectious virus particles and the number of virus genome copies detected by quantitative PCR is not constant, as genetic material from inactivated viruses can be detected and the proportion of non-infectious particles may vary between samples. Finally, there are issues of variability and reproducibility in nucleic acid-based testing in different laboratories and the efficacy of extraction from the oyster food matrix, which make accurately quantifying infectious norovirus extremely challenging. For this reason, establishing legal thresholds or guidelines based on norovirus RNA levels in foods is not straightforward.

One of the food items commonly associated with norovirus infection is bivalve molluscs, in particular oysters, as these are commonly consumed without cooking. The oysters are grown in beds that can be contaminated from sewage effluent being washed in via failures of the sanitation system, for example the introduction of untreated sewage into areas used for shellfish production, following extreme rainfall events (e.g. Le Guyader *et al.*, 2010). Filter-feeders such as oysters are then able to bio-accumulate the virus in their digestive tract (Rajko-Nenow *et al.*, 2012).

This strategic risk assessment will focus on levels of norovirus in two species of oyster cultivated in the UK: the pacific oyster, *Crassostrea gigas,* and the native oyster, *Ostrea edulis*. The human population of concern for this risk assessment is the general UK population that consumes raw oysters.

3. Hazard characterisation

3.1 Disease characterisation

The common symptoms of norovirus include projectile vomiting and watery non-bloody diarrhoea. Rarer symptoms include fever, headaches, stomach cramps and aching limbs. The incubation period is around 12-48 hours, and the disease is usually self-limiting, with symptoms lasting for one to three days. Symptoms can be more prolonged in vulnerable groups. Severe intestinal disease such as inflammatory bowel disease, necrotising enterocolitis and seizures can occur in neonates or young children in very rare cases. In elderly, malnourished or immunocompromised groups, excessive and prolonged vomiting and diarrhoea can lead to dehydration, malnutrition, or death (Ludwig-Begall, Mauroy and Thiry, 2021).

Based on adult volunteer studies, it is estimated that 15% to 35% of norovirus infections are asymptomatic, which may have resulted from acquired immunity or genetic resistance (CDC, 2017). An estimated 20% of the European population is genetically less-susceptible to norovirus infection (Nordgren *et al.*, 2016).

Norovirus RNA is shed in high concentrations in the faeces of infected individuals, with peak viral titres varying between 10^5 - 10^9 genome copies/g of faeces, and can be shed for up to 8 weeks (with a median of four weeks of post clinical shedding after symptoms have subsided (Ludwig-Begall, Mauroy and Thiry, 2021). Norovirus RNA has been detected in symptomatic and asymptomatic individuals and is commonly found in wastewater treatment effluents, particularly over the winter months (Rajko-Nenow *et al.*, 2013).

The GII norovirus genogroup accounts for the majority of all genotypes associated with human outbreaks, in particular genotype GII.4 (Bull *et al.*, 2006). Since 2012, the dominant strain worldwide is GII.4 Sydney (CDC, 2022). Genogroup I (GI) strains co-circulate in the human population and are regularly involved in foodborne cases (Thebault *et al.*, 2013), sometimes in higher proportions than GII strains (Verhoef *et al.*, 2015). A study of outbreaks reported from different regions has shown that GI strains are over-represented in shellfish-related outbreaks relative to the proportion in norovirus infections from all sources (Le Guyader, Atmar and Le Pendu, 2012). This may be

because GI strains bioaccumulate more effectively in oyster digestive tissue than GII strains (Le Guyader *et al.*, 2008; Zakhour *et al.*, 2010; Maalouf *et al.*, 2011).

Norovirus is typically a very common and mild illness. The UK hospitalisation rate for foodborne norovirus was estimated to be 0.6% (Holland and Mahmoudzadeh, 2020). The most frequent long-term sequelae associated with norovirus infection is chronic diarrhoea and occurs most commonly in immunocompromised and younger patients (Petrignani *et al.*, 2018). In the UK, a study found that 1.5% of patients admitted to hospital with norovirus had chronic diarrhoea (Petrignani *et al.*, 2018), bearing in mind the hospitalisation rate of 0.6%. Other symptoms such as dyspepsia and constipation also associated with viral gastroenteritis was transient and cases improved after 6 months (CDC, 2017).

3.2 Cases and outbreaks in the UK

Between 2009 – 2018, there were an average of 10,084 confirmed laboratory reports of norovirus in the UK each year (Holland and Mahmoudzadeh, 2020). Despite this relatively low value, norovirus is the most common cause of infectious intestinal disease in the UK. An FSA-funded quantitative microbiological risk assessment (QMRA) estimated the proportion of foodborne norovirus cases in the UK at 16%, although with significant uncertainty (O'Brien *et al.*, 2019); an FSA technical review of this model suggested the median estimate could be as low as 12%, but that uncertainty remained very high (Gherman *et al.*, 2019), and an individual-based modelling approach based on some of the same sources (O'Brien *et al.*, 2019) estimated this as high as 35%. Many cases are likely to go unreported **(uncertainty)**, due to the often-mild symptoms and short illness duration, as well as NHS advice to avoid hospitals while infectious. For every case reported to national surveillance, it is estimated that there are 13 GP consultations and 288 community cases (Tam *et al.*, 2012). An FSA economic study calculated the annual cost of foodborne norovirus to be £1.7 billion (Daniel *et al.*, 2018).

Between 2013 – 2022 there have been 28 suspected viral or confirmed norovirus outbreaks that have descriptive epidemiological links to consumption of oysters in England, giving an average of 2.8 outbreaks per year (UKHSA, 2023). Between 2017 – February 2023 there have been 8 suspected viral or confirmed norovirus outbreaks that have descriptive epidemiological links to consumption of oysters in Scotland (this includes outbreaks which also involved English cases), giving an average of 1.1

outbreaks per year (FSS Incidents, 2023) (see Table 1 and Figure 1). Data from Wales is not routinely collected and data from Northern Ireland was not available.

Table 1: The number of human cases in norovirus outbreaks linked to oyster consumption, recorded by month of onset of the first case, between 2013 and 2022 in England (UKHSA, 2023) and between 2017 and February 2023 in Scotland (FSS Incidents, 2023)

Reporting year	**Number of Human Cases** **England (Scotland)**	**Number of outbreaks** **England (Scotland)**
2013	222 (NA)	7 (NA)
2014	43 (NA)	5 (NA)
2015	17 (NA)	1 (NA)
2016	100 (NA)	1 (NA)
2017	90 (18)	2 (3)
2018	106 (24)	5 (1)
2019	58 (0)	1 (0)
2020	157 (187)	2 (0)
2021	449 (0)	3 (0)
2022	65 (0)	1 (0)
2023	NA (30)	NA (4)
Total	**1307 (259)**	**28 (8)**

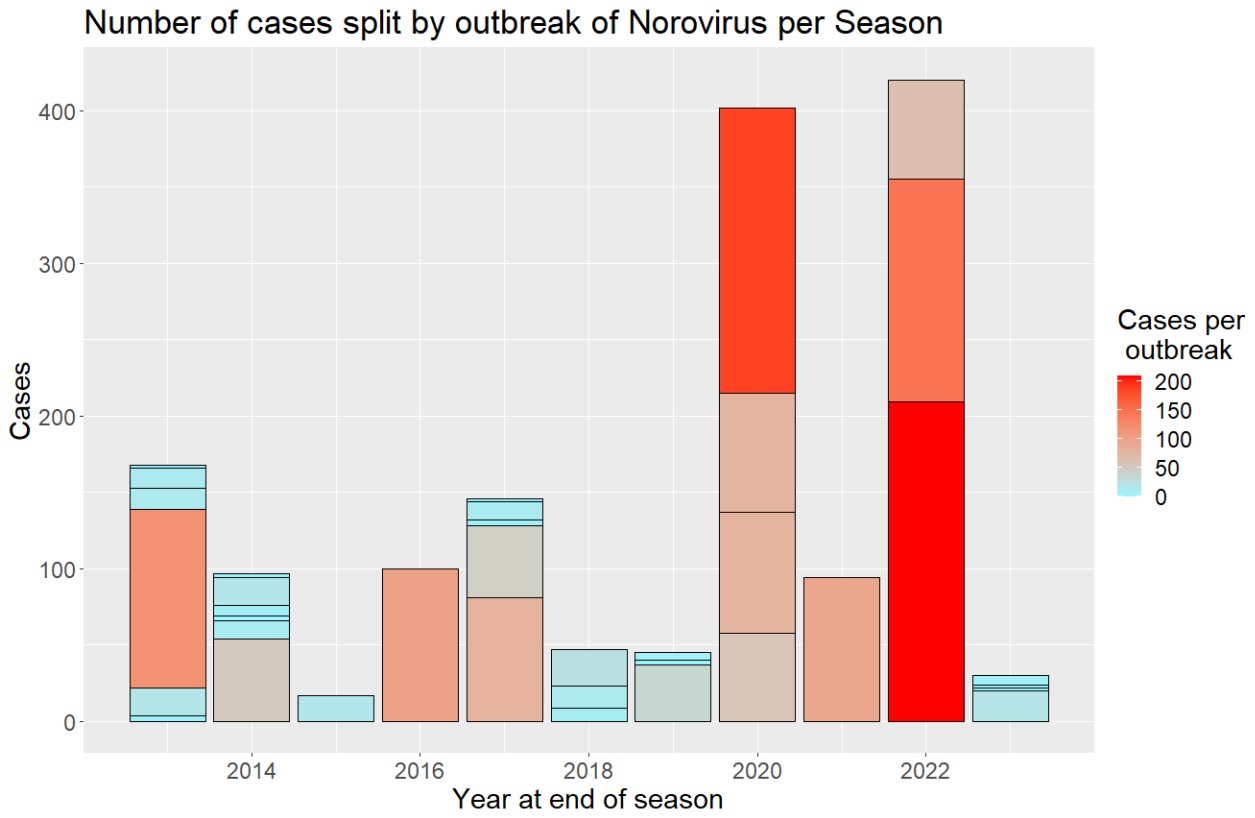

Figure 1: Number of outbreaks linked to oysters in England (between 2013-2022) and Scotland (between 2017-February 2023). 2023 only includes cases from Scotland in the first 2 months. The bars represent the number of cases per season broken down into the number of cases per outbreak, which are represented by the colours in the key. Reported outbreaks are linked to oysters using descriptive epidemiology and were either suspected or confirmed as norovirus. A season is from July to the June of the following year e.g. July 2018 to June 2019.The season is the season of symptom onset in the first case.

In England, between 2013 – 2022, there were 1,307 reported cases of norovirus infection linked to oysters (UKHSA, 2023). In Scotland, between 2017 – 2023, there were 259 reported cases of norovirus linked to oysters. Cases are more frequent during winter months (see Figure 2). It is possible that underreporting of norovirus outbreaks linked to oysters is smaller than general underreporting - oysters carry a well-known norovirus risk - such outbreaks are more likely to be investigated than norovirus outbreaks caused by other foods **(uncertainty)**. Hassard *et al.*, 2017, estimated that between 14,593 and 30,160 cases of norovirus in the UK were caused by contaminated shellfish. Assuming around 4.4 million oyster meals are consumed yearly in the UK (see section 4.3), this is

similar to modelling done by an FSA-funded norovirus attribution study, which suggested that around 1 in 160 oyster meals results in norovirus infection (Gherman *et al.*, 2019).

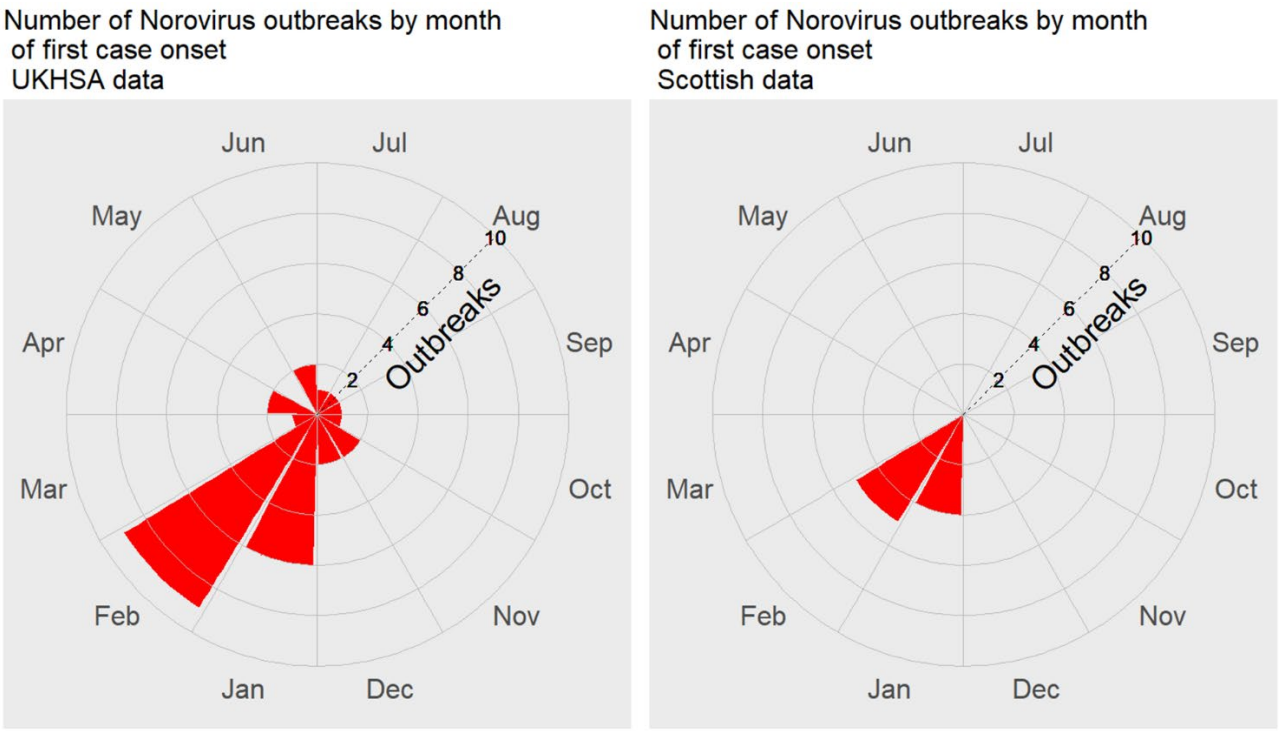

Figure 2: Rose plot of the number of norovirus outbreaks linked to oyster consumption, recorded by month of onset of the first case, between 2013 and 2022 in England (UKHSA, 2023) and between 2017 and February 2023 in Scotland (FSS Incidents, 2023).

The FSA-funded Norovirus Attribution Study (NoVAS) estimated that 3% of foodborne norovirus cases in the UK were attributable to the consumption of oysters (O'Brien *et al.*, 2019). An international review of outbreak reports estimated that between 2000-2007, bivalve shellfish accounted for 17.5% (7/40) of all foodborne norovirus outbreaks (Baert *et al.*, 2009). Similarly, it has been reported that the percentage of norovirus outbreaks associated with seafood is around 10-20% in countries where seafood is eaten raw (Terio *et al.*, 2010). This difference between estimates of the proportion of overall foodborne cases attributed to oysters and of outbreak-related cases to oysters could be due to investigator bias because of the long-established association between seafood and norovirus (O'Brien *et al.*, 2019).

The FSA recorded 110 food incidents associated with consumption of oysters and potentially linked to norovirus between 2000 and 2022 (although data pre-2010 is sparse). Reports from different FBOs using the same supplier (and potentially part of the

same outbreak) are sometimes treated as separate incidents which could lead to an over estimation of incidents **(uncertainty)**. FSS has recorded 16 food incidents associated with oysters grown or eaten in Scotland and potentially linked to norovirus between 2017 and February 2023 (FSS Incidents, 2023).

The causative pathogen of outbreaks epidemiologically linked to oysters is not always identified, often due to lack of faecal samples from cases for laboratory confirmation. Other enteric viruses such as Aichi virus, astrovirus, enterovirus, sapovirus and rotavirus have symptoms that are indistinguishable from norovirus (Le Guyader *et al.*, 2008) and may contribute to illness following oyster consumption **(uncertainty)**. For instance, when stool samples from 12 patients with gastroenteritis following oyster consumption were tested, 9 were positive for norovirus, 6 positive for Aichi virus, 6 positive for enterovirus, 3 for astrovirus and 2 for rotavirus (some stool samples had multiple viruses present in one sample) (Le Guyader *et al.*, 2008). A total of 7 norovirus genotypes were identified, including 5 stool samples contaminated by 2 different norovirus strains. Norovirus, Aichi virus, astrovirus and rotavirus were also detected in 2 oyster samples left over from consumers.

In England, 2 outbreaks were recorded where cases were coinfected with sapovirus or *Clostridium perfringens*, as well as norovirus (UKHSA, 2023). These viruses have no infectivity assay associated with them at present. Some of these enteric viruses, particularly Aichi virus and sapovirus, may also have the potential to bind and bioaccumulate within shellfish **(uncertainty)** (Le Guyader, Atmar and Le Pendu, 2012).

Sewage will contain norovirus (and other viruses) from multiple contamination sources, typically resulting in multiple genogroups contaminating a single bed or oyster. Person-to-person spread or spread through contamination of food, by food handlers, will more likely result in cases in a single restaurant infected with a single norovirus strain. Genotyping can give an indication of whether the source of an outbreak is likely to be oysters, as can epidemiological evidence of multiple cases of illness in different restaurants/settings linked to the same batch of oysters.

3.3 Dose response

Norovirus particles are thought to be highly infectious (Teunis *et al.*, 2008; Lopman *et al.*, 2012; Atmar *et al.*, 2014) though it is not possible to directly measure the infectivity of wild-type virus with cell assays that are typically used in other viral infectivity studies.

Human susceptibility to norovirus is strongly linked to the presence of histo-blood group antigens on the epithelial cell surfaces. Individuals with these antigens are known as 'secretors' and are susceptible to norovirus illness, while non-secretors are resistant to challenge (Lindesmith *et al.*, 2003; Nordgren and Svensson, 2019). Non-secretors are rarely infected by GII.4 noroviruses, however they are susceptible to other norovirus strains and can become ill symptomatically or asymptomatically and could potentially still shed virus after ingestion (Nordgren *et al.*, 2016). Non-secretors lack the receptor which allows the virus to bind and infect intestinal cells, whereas, secretors exhibit the phenotype which allows the virus to bind (Teunis *et al.*, 2008). The histo-blood group antigens appear to facilitate entry of the virus into human cells, although the precise mechanism is unknown. A study estimated that 80% of the European population has secretor status, with the remaining 20% significantly less susceptible to norovirus infection (Nordgren *et al.*, 2016).

Those who have had norovirus can develop acquired immunity, which makes them less susceptible to reinfection, particularly by similar norovirus strains and at lower doses (Simmons *et al.*, 2013). Studies on acquired immunity find that the duration of immunity post-infection ranges from 2 months (Parrino *et al.*, 1977) to 9 years (Simmons *et al.*, 2013).

Human volunteer trials that have been carried out to estimate the norovirus infectious dose are summarised in Section 8.3. A dose-response relationship exists between the amount of genome ingested and the probability of human illness, with higher doses leading to an increased probability of infection and illness.

The infectious dose is the dose required to infect 50% of participants and it varies in different studies from around 1,000 to 500,000 genome copies, with modelling studies suggesting it may be even lower (Teunis *et al.*, 2008, 2020; Leon *et al.*, 2011; Atmar *et al.*, 2014; Van Abel *et al.*, 2017; Rouphael *et al.*, 2022) (see Table 2). This variation may be due to reasons such as different calculations used by researchers for the infectious dose, or variation in the infectivity of different norovirus strains. Symptomatic illness

occurs in a majority, but not all, of infected people (Teunis *et al.*, 2008; Atmar *et al.*, 2014; Rouphael *et al.*, 2022).

There is no dose-response value for vulnerable groups due to ethical concerns. Modelling studies often extrapolate the patterns seen in higher doses by predicting a high infectivity at low norovirus doses; this varies depending on whether the model is foodborne or waterborne and it is unknown how much represents infectious virus (Van Abel *et al.*, 2017). Therefore, providing accurate predictions is challenging and the uncertainty at low doses remains high.

Table 2: Results from human volunteer trials to measure the dose required to infect 50% of susceptible participants.

Study	**Dose required to make 50% participants infectious or ill (genome copies)**	**Calculated or observed?**
Teunis *et al.*, 2008	1,015	calculated
Leon *et al.*, 2011	10,000	observed
Atmar *et al.*, 2014	2,800	calculated
Rouphael *et al.*, 2022	510,000	calculated

Studies assessing the levels of norovirus in oysters epidemiologically linked to illness are discussed in section 4.2.

3.4 Detection methods

The internationally recognised method for the extraction and quantification of norovirus genetic material from food is ISO 15216-1:2017[2] (International Organization for Standardization, Geneva, 2017). Target sequences within the viral RNA are amplified by quantitative reverse transcription PCR (qRT-PCR). Other tests exist for norovirus, including detection assays for the intact viral capsid (O'Brien *et al.*, 2019), and microscopy-based tests (Chung *et al.*, 2021), as well as other PCR-based methods which differ from ISO 15216 (Le Guyader *et al.*, 2008).

The steps involved in one typical norovirus quantification method are briefly described below. Other methods may vary somewhat but the general order to steps will remain the same. Detection typically takes 24 hours from sampling to test result, but these times will vary between labs.

Sample processing

Oyster samples are gathered, and the digestive tissue is excised, pooled (for each sample, a minimum of ten oysters are selected) and chopped. A control virus (often Mengo virus) is used and the supernatant from infected cell culture is added to the sample as a control, to determine the efficiency of the extraction process. The processed sample can be kept at 4°C for up to a month prior to testing (Lowther *et al.*, 2018).

RNA extraction

Total RNA is extracted using magnetic extraction reagents and extraction machinery. Viral RNA is extracted by lysis with guanidine thiocyanate and adsorption on silica. A negative control of water is used and tested in parallel with each set of samples extracted. Eluted RNA can be stored at -20°C until needed (Lowther *et al.*, 2018).

[2] Referred to as ISO 15216 within this report. ISO 15216-2:2017 specifies the method for norovirus detection, does not quantify norovirus genome copies, and is not considered further in this risk assessment.

One step qRT-PCR

Norovirus GI and GII genogroups and the virus control have specific and distinct primers and probes associated with them. The qRT-PCR assays are prepared containing a mix of primers, probes, and enzymes before amplification on a real-time PCR machine. Wells containing nuclease free water and the above qRT-PCR reaction mixes are included on each plate as a negative control (Lowther *et al.*, 2018).

Quantification

Quantification is carried out using a log dilution series of linear double stranded DNA molecules carrying the GI and GII target sequences. Comparison of measured control virus with a standard curve is used to calculate the extraction efficiency. qRT-PCR inhibition is measured with RNA external controls (Lowther *et al.*, 2018).

Samples with an extraction efficiency of 1% or less are discarded from analysis. Samples with PCR inhibition of 75% or more are discarded from analysis.

The results are not corrected for the extraction efficiency calculated from the virus process control, or the PCR inhibition values. While the use of a control virus can give a general indication of whether the efficiency of the extraction is acceptable or not, the virus as applied is not a good proxy for norovirus in other ways – for instance, it is pipetted onto the oyster samples immediately before extraction, while norovirus present as a result of contamination in the environment may be bound to the surfaces of tissues within the interior of the digestive gland, and it is unknown how well its inactivation corresponds to norovirus inactivation.

Samples with lower copy numbers will have more uncertainty due to the stochastic nature of PCR when few RNA copies are present.

3.4.1 Testing capacity of laboratories

Official/ commercial testing using accredited ISO 15216

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) is the National Reference Laboratory (NRL) for Foodborne Viruses in GB. From communication with the FSA Scientific Sampling and Laboratory Policy team, Cefas is the only official laboratory in the UK that is accredited to test for norovirus in bivalve mollusc digestive glands with

ISO 15216. Cefas has recently started to offer their service commercially; however, their commercial capacity is low, at approximately 30 samples a month (FSA Scientific Sampling and Laboratory Policy, 2023).

The Austrian Agency for Health and Food Safety is the NRL for Foodborne Viruses in NI.

The Marine Institute is the only laboratory in Ireland that is accredited to test norovirus in bivalve molluscs, based on the method outlined in ISO 15126 (Keaveney, 2023).

Official/ commercial testing in labs not accredited for ISO 15216

Laboratories may have the capability to test for norovirus, but not the accreditation for ISO 15216. Government laboratories such as the United Kingdom Health Security Agency (UKHSA) and commercial laboratories such as Neogen and Campden BRI may offer norovirus testing in food but are not currently accredited for it. There are many labs that are able to use qRT-PCR or RT-PCR techniques to test for viruses, but generally in non-food matrices (UKAS website).

Laboratories may offer quantitative norovirus testing and provide results in terms of cycle threshold (Ct) values. The Ct value is the number of cycles needed to reach a certain fluorescent threshold value (set for every individual PCR experiment, based on certain criteria). The Ct value can broadly categorise the concentration of viral genetic material in a food matrix and is particularly useful when comparing two samples that have been run in exactly the same RT-PCR experiment, as it can determine which has more DNA (or RNA). A lower Ct value indicates a higher concentration of DNA. A Ct value alone cannot be used to determine the level of norovirus in an oyster sample and needs to be compared to reference samples of known norovirus levels which have been measured in the same experiment. A Ct value on its own cannot be accurately compared to historical values from different experiments, even if they are from the same oyster bed and tested by the same laboratory.

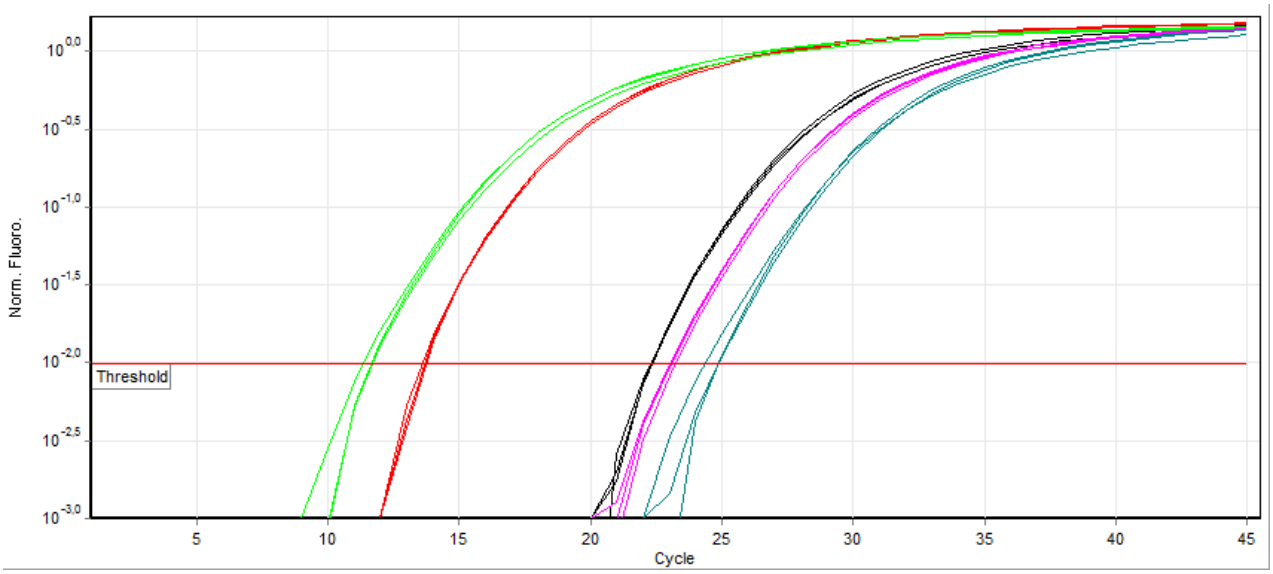

Figure 3: An example of a PCR experiment with the sample curves. A new fluorescence threshold on the y axis is set for every individual PCR experiment, based on certain criteria. The Ct values are read for each sample as the cycle number where the curve meets the threshold. The sample with the lower Ct value has more DNA as it takes fewer cycles to reach a defined fluorescence threshold. The threshold is usually adjusted for each experiment based on the shape of the amplification plot. This is why results from different experiments are not comparable.

The FSA Scientific Sampling and Laboratory Policy team recommend the use of accredited laboratories for any analysis required for official controls or official activities. Accreditation to ISO 17025 provides assurance that the labs are competent, and results are more consistent with other labs (FSA Scientific Sampling and Laboratory Policy, 2023). However, unaccredited qRT-PCR based methods from competent laboratories can still provide valuable information, and comparable values, given appropriate extraction methods are used for the shellfish and if samples are compared to norovirus controls within the same PCR experiment. It is recommended that oysters are pooled for norovirus enumeration, as individual oysters may have highly variable levels of the virus, however, sometimes in outbreak situations it is necessary to test fewer animals as that may be all that is available. In this case a result can be generated, but it is less representative of the oyster batch as a whole.

3.4.2 Interlaboratory differences

Limit of detection and quantification

The limit of detection (LOD) is the lowest concentration of target in a test sample that can be reliably detected with 95% confidence (EFSA Panel on Biological Hazards (BIOHAZ), 2012).

The limit of quantification (LOQ) is the lowest concentration of target material in a test sample that can be quantitively determined with acceptable precision and accuracy, under experimental conditions specified in the method (EFSA Panel on Biological Hazards (BIOHAZ), 2012).

There was some variation in the LOD and LOQ between laboratories using ISO 15216 as LOD and LOQ are not inherent to the method but need to be determined experimentally by each laboratory. The majority of papers referenced in Annex I had LOD values as 20 copies/g or 40 copies/g and LOQ values of 100 copies/g (see Table 3). A European survey of production areas and dispatch centres had 13 participating laboratories, with LOD ranging from 13 to 264, and LOQ ranging from 40 to 389 copies/g (European Food Safety Authority, 2019). This variation was thought to be due to differences in chemical composition and inhibitors in local oysters, laboratory strains of norovirus, PCR machines, and the effects of extremely high or low amounts of viral RNA.

Table 3: LOD and LOQ values reported for literature studies identified in the search. The method used was the ISO 15216 or a similar quantitative method.

LOD, copies/g	LOQ, copies/g	Reference
-	100	(Flannery, Keaveney and Doré, 2009)
13	-	(Baker *et al.*, 2011)
40	100	(Cefas, 2011)
40	100	(James A. Lowther *et al.*, 2012)
20	-	(Rajko-Nenow *et al.*, 2012)
20	-	(Rajko-Nenow *et al.*, 2013)
-	70	(Schaeffer *et al.*, 2013)
20	100	(Rajko-Nenow *et al.*, 2014)
40	100	(Campos *et al.*, 2015)
-	70	(Loury *et al.*, 2015)
40	100	(Lowther *et al.*, 2018)

LOD, copies/g	LOQ, copies/g	Reference
-	100	(Rupnik *et al.*, 2018)
For GI, the LOD ranged between 13 and 264 copies. For GII, the LOD ranged between 20 and 196 copies.	For GI, the LOQ ranged between 40 and 298 copies. For GII, the LOQ ranged between 75 and 389 copies.	(European Food Safety Authority, 2019)
-	100	(Lowther, Cross, *et al.*, 2019)
20	100	(Hunt *et al.*, 2020)
-	LOQ was 140 for GI and 130 for GII.	(Battistini *et al.*, 2021)
The LOD95 was 18 for NoV GI.2 and 61 for GII.4	The LOQ was determined at 30 for NoV GI.2 and 61 for GII.4	(Dirks *et al.*, 2021)
20	100	(Rupnik *et al.*, 2021)
20	100	(Keaveney *et al.*, 2022)

The current LOD and LOQ determined by Cefas are 71 copies/g and 184 copies/g, respectively for ISO 15216 (Lowther, 2023).

In the literature, samples are typically assigned the value of LOD/2 when norovirus is 'not detected' and LOQ/2 when they are not quantifiable but positive.

Intra- and interlaboratory variability

Repeatability and reproducibility of the ISO 15216 method was assessed by 10 international laboratories (International Organization for Standardization, Geneva, 2017; Lowther *et al.*, 2019) and these were used to set the repeatability and reproducibility limits which are expected within the same laboratory, and between different laboratories. The repeatability limit described in ISO 15216 states that identical material tested in the same laboratory by the same operator and equipment should not vary by more than 0.60 $\log_{10}$ copies/g (or 3.98-fold) in 95% of cases. The reproducibility limit described in ISO 15216 states that identical material tested in a different laboratory with different operators and equipment should not vary by more 1.35 $\log_{10}$ copies/g (or 22.39-fold) in 95% of cases.

To put this into context, a test result of 3 log_{10} norovirus genome copies/g of oysters when replicated in the same laboratory should be between 3.00 – 0.60 = 2.40 log_{10} copies/g and 3.00 + 0.60 = 3.60 log_{10} copies/g. The same material, when enumerated in a different laboratory, should be between 3.00 – 1.35 = 1.65 log_{10} copies/g and 3.00 + 1.35 = 4.35 log_{10} copies/g.

Expressing the variability in a different way, a test result of 1,000 norovirus genome copies/g of oysters when replicated in the same laboratory should be between 1,000/4 = 250 copies/g and 1,000 x 4 = 4,000 copies/g. The same material, when enumerated in a different laboratory, should be between approximately 1,000/20 = 50 copies/g and 1,000 x 20 = 20,000 copies/g.

The higher the norovirus levels, the more aligned intra- and interlaboratory results are.

The results tend to vary more between laboratories due to differences in equipment e.g., the PCR reagents, the extraction method, the real-time PCR machine used; and personnel carrying out the experiment.

Method efficiency

In ISO 15216, the extraction efficiency from shellfish is assessed by spiking samples with a process control virus (often Mengo virus) of a known concentration and measuring its levels after the extraction process. According to ISO 15216, oyster samples with >1% extraction efficiency are acceptable. Additionally, quantification of norovirus in shellfish can be subject to problems with inhibition of RT-PCR by the shellfish tissue components. ISO 15216 considers qRT-PCR inhibition <75% as acceptable.

Reported efficiency and inhibition ranges are given in Table 4 for studies using ISO 15216. Other publications do not report the extraction efficiency or qRT-PCR inhibition, simply stating that they are within the tolerances required. Studies do not correct their reported quantitative results for these inefficiencies.

Table 4: Reported extraction efficiency ranges and PCR inhibition ranges for studies using ISO 15216

Study	Extraction efficiency Mean	Extraction efficiency Range	PCR inhibition Mean	PCR inhibition Range
Keaveney *et al.*, 2022	7.8%-10.8%	1%-38.3%	8.9%-22%	0%-74.1%
Pavoni *et al.*, 2022	42.9%	1.24%-100%	NS	NS
Lowther *et al.*, 2018	28.7%	1.1%-99.6%	14%	0%-74.3%
Lowther *et al.*, 2011	13.2%	1%-78.4%	16.6%	0%-75%

Other qRT-PCR-based methodologies report extraction efficiencies of 10% based on a feline calicivirus process control (Lowther, Henshilwood and Lees, 2008).

Experiments similar to ISO 15216 carried out by Le Guyader *et al.*, (2008) were more stringent, only accepting samples with less than 50% inhibition for further analysis. Dilution of the sample was used to reduce the PCR amplification efficiency in some studies (Rajko-Nenow *et al.*, 2012; Dirks *et al.*, 2021).

4. Exposure Assessment

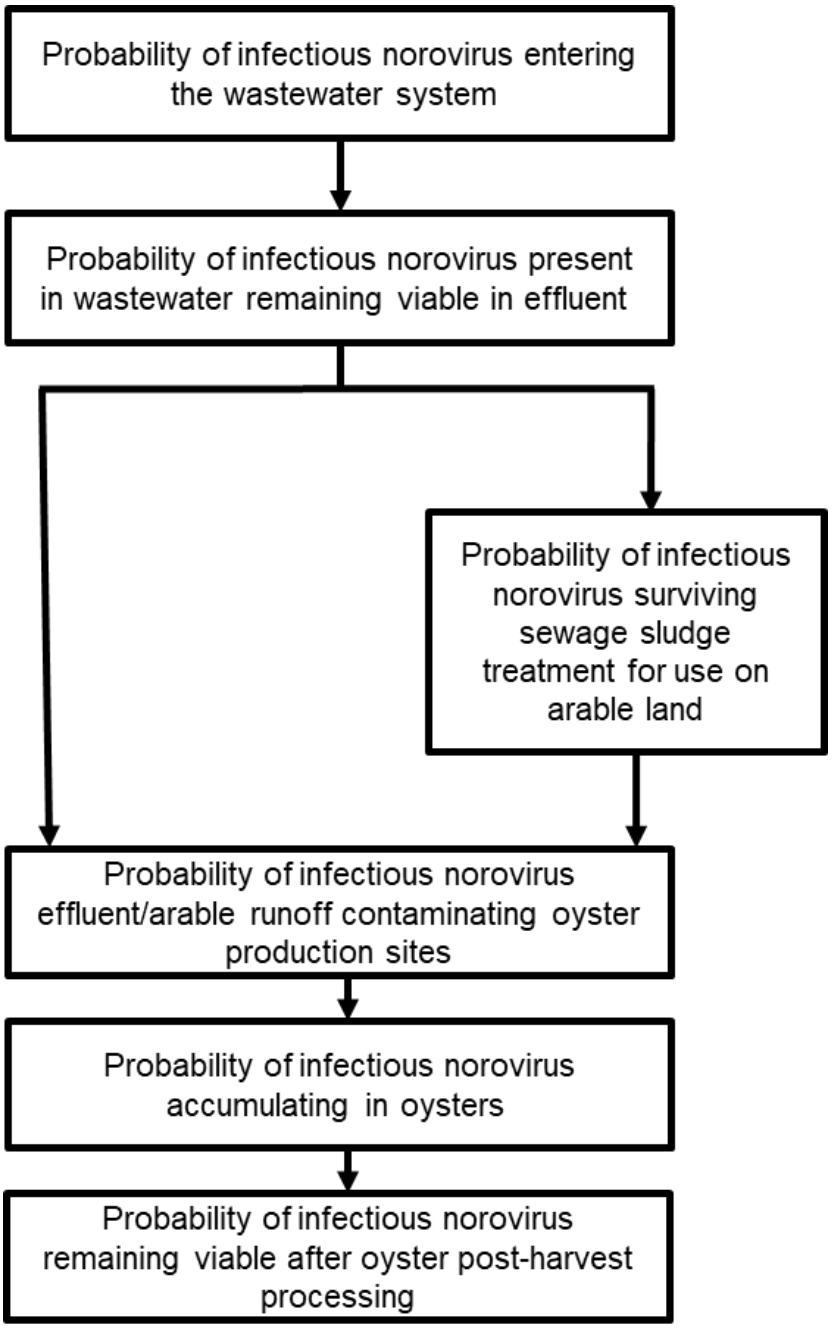

Figure 4 The risk pathway for the probability of infectious norovirus being found in oysters, further discussed in Section 4.1.

4.1 Contamination of oyster sites

Wastewater discharges into the sea have been shown to contain high concentrations of norovirus and when sewage treatment processes are bypassed, as can happen with extreme weather events such as flooding, contamination of shellfish-growing beds with

raw sewage containing the virus from faecal material can occur (Le Guyader et al., 2008; Keaveney et al., 2022). Wastewater treatment is not completely effective at eliminating norovirus in water effluents, with a reduction of around 1-2 $\log_{10}$ copies in final effluent compared to crude sewage (Cefas, 2011). Exposure to multiple low doses of effluent or an equivalent single major event appear to have the same effect on accumulation of norovirus in oysters (Ventrone *et al.*, 2013).

Viral particles can survive for a long period of time in the water column. This is particularly true in cold waters (Duizer *et al.*, 2004). Shellfish are filter feeders, which means they can bio-accumulate the virus within their digestive tissues from the surrounding water (Rajko-Nenow et al., 2013). Oysters have been shown to accumulate virus up to 2 $\log_{10}$ (100 fold) more compared to the surrounding waters (Burkhardt and Calci, 2000). Specific binding of norovirus to shellfish digestive tract has been suggested as a contributing factor in the delayed depuration of norovirus compared to bacteria (Le Guyader *et al.*, 2008). Le Guyader *et al.*, (2008), found that norovirus can potentially remain at an infectious level in oysters for around 3 weeks. The authors found that after an initial outbreak, associated with a flooding event in a French shellfish bed, norovirus levels did not decrease until week 3 of sampling. Concentrations in shellfish decreased from a mean concentration of 7 x 10^3 genome copies/g for GI and 1.3 x 10^3 genome copies/g for GII, to an average of 200 RNA copies/g for each genogroup.

Risk factors linked to norovirus contamination of shellfish waters include the size of water catchment areas, discharges to the catchment/estuary, human population in the catchment (Cefas, 2015), harvesting area classification, *E. coli* levels and low temperatures (James A Lowther *et al.*, 2012) and occurrence of stormwater discharges (Campos *et al.*, 2016).

E. coli is a gastrointestinal bacterium, and its presence in water can signify a risk that pathogens of faecal origin may be present; repeated detection highlights an increased food safety risk. *E. coli* detection is used as a proxy in monitoring the sanitary quality of oyster beds, as recommended by CODEX Standard for Live and Raw Bivalve Molluscs (CXS 292-2008). While bacterial indicators do not provide an effective indicator for norovirus in individual oysters (O'Brien *et al.*, 2019), there is a significant correlation between *E. coli* and norovirus levels on a production-site basis (Cefas, 2011). Because norovirus binds to the oyster flesh and therefore remains associated for a much longer time period than *E. coli*, there may be a correlation in levels at the point of contamination,

but the rate of clearance is different, leading to a much weaker association in the longer term.

The association between *E. coli* levels and norovirus may be weaker in shellfish beds in remote areas, where the source of each microorganism is different. A project assessing the risk factors for norovirus in shellfish in a loch off the western coast of Scotland found a weak correlation between winter *E. coli* and norovirus tissue GI and GII levels at one site (site B) and no correlation at another site (site A) (Magill *et al.*, 2008). One explanation for this is that the area in western Scotland where the survey took place is sparsely populated, with the *E. coli* found coming primarily from farming run-off from the surrounding landscape rather than human *E. coli* from sewage effluent.

A comparison of the prevalence of norovirus RNA in oysters from Australia and the UK found that prevalence is higher in the UK than in Australia, and is intricately linked to the sanitary quality of the waters (Food Standards Australia New Zealand, 2017).

4.1.1 Seasonality

Various studies have indicated that oyster contamination shows strong positive correlations with colder seasons, notably winter, thought to be linked to reduced degradation of the virus due to colder seawater temperature (EFSA Panel on Biological Hazards (BIOHAZ), 2012; James A. Lowther *et al.*, 2012; Cefas, 2015; Battistini *et al.*, 2021) and a spike in human cases in winter, characteristic of the disease (Atmar and Estes, 2006). For instance, a two year survey of oyster production areas in the UK observed 90% positivity in winter months (October to March) compared to 62.4% positivity in summer months (April to September) (James A. Lowther *et al.*, 2012).

4.1.2 Classification of shellfish beds

Shellfish production areas are classified according to the levels of *E. coli* detected in the shellfish (see Table 5).

Table 5: Classification of shellfish production areas (FSA, 2023).

Bed class	Minimum # of samples per year	Sample requirements	Maximum sample result
Class A	10	80% of sample results ≤ 230 *E. coli*/100g	700 *E. coli*/100g
Class B	8	90% of sample results ≤ 4,600 *E. coli*/100g	46,000 *E. coli*/100g
Class C	8	All sample results ≤ 46,000 *E. coli*/100g	All sample results ≤ 46,000 *E. coli*/100g

According to Annex III of retained EU Regulation No 853/2004 (GB) and EU Regulation No 853/2004 (NI), oysters collected from class A production areas can be sold for direct human consumption. Oysters from class B areas must undergo depuration or relaying before sale for human consumption. Oysters from class C areas must first undergo a long period of relaying before being placed on sale for human consumption. Oysters from class B or C areas may be sterilised in hermetically sealed containers or heat-treated instead of relayed/depurated.

The majority of oyster norovirus contamination occurs via water whilst growing in oyster beds, however in catering and retail establishments, food handlers can theoretically also be a source of norovirus infection (O'Brien *et al.*, 2019).

4.1.3 Effective interventions

Norovirus is unable to replicate within the shellfish. There are a number of interventions that could reduce the levels of norovirus in oysters, but these are out of scope and only mentioned briefly here.

Cooking oysters will reduce the viral load, however, oysters are commonly eaten raw and not likely to be cooked so interventions with heat treatment are less likely (Bartsch *et al.*, 2019).

Depuration with sterile seawater, a method that flushes out bacteria within days or hours, is less effective at removing viral particles, which are much smaller, and has not been proven to eliminate norovirus from oysters, as summarised in an FSA-funded review (McLeod, Polo, Le Saux and Françoise S Le Guyader, 2017). Minimum time and

temperature are not stipulated in Retained EU Regulation No 853/2004 (GB) or EU Regulation No 853/2004 (NI) for commercial depuration. However, depuration at elevated temperatures of 15-17ºC for at least 4 days has been shown in some studies to significantly reduce norovirus levels and lead to an absence of illness associated with 50,000 oysters from contaminated beds (Doré *et al.*, 2010; Rupnik *et al.*, 2021).

Long-term relaying (for a month) of oysters in sites with lower contamination has also been shown to reduce levels of norovirus (McLeod, Polo, Le Saux and Françoise S. Le Guyader, 2017; Battistini *et al.*, 2021). All treatments may have an economic impact, but we have not assessed this.

Noroviruses are resistant to inactivation by low pH, low temperatures (e.g. freezing) and some common disinfectants (Nims and Plavsic, 2013; Barclay *et al.*, 2014).

Food businesses need to continuously consider extrinsic environmental factors that may affect the safety of coastal sites where oyster beds are located, such as rainfall levels, average wind speed and direction, and nearby locations of potential sewage release points. These factors influence the water quality which oysters will be exposed to, and food businesses may need to consider these factors in their food safety management systems.

4.2 Quantitative data on norovirus in oysters

Several large-scale sampling schemes for oysters have been carried out in the UK and Ireland. These data have been collected and presented in this section as ‘baseline data’. We compare these with the levels of norovirus in oysters from batches that have been epidemiologically linked to outbreaks, to understand whether outbreak batches have significantly higher norovirus levels, and whether it is possible to use these quantitative data to assess risk.

The presence of norovirus in oysters has been shown to be significantly correlated to the illness reporting rate (number of illnesses divided by number of portions) (Lowther *et al.*, 2010). The relationship between illness and norovirus levels in oysters was less straightforward. In this study norovirus levels were generally quite low, with the exception of a single highly contaminated batch. When the outlier batch was removed from analysis, the levels of norovirus within batches did not significantly correlate with the illness reporting rate (Lowther *et al.*, 2010). If the outlier batch was included, a significant

correlation was seen. The outlier was a batch of oysters with 14 times higher levels of norovirus than the average contaminated batch, which caused half the cases of illness within the study period. The illness-reporting rate for positive batches was 0.28%, and the authors commented that detection of norovirus at low levels did not necessarily lead to illness. A further study by the same authors found a statistically significant difference in norovirus levels between outbreak-related oyster samples and controls, with geometric means reported as 1,048 copies/g and 121 copies/g, respectively (James A Lowther *et al.*, 2012)

An investigation into an outbreak in a nursing home in France observed a dose-response relationship between the quantity of oysters consumed and acute gastroenteritis. It was suggested that a minimum infectious dose of <100 genome copies was needed, however, as this was a vulnerable group, the dose suggested is likely less than is needed to cause illness in the general population (Loury *et al.*, 2015).

4.2.1 Norovirus in oysters at retail

We chose baseline norovirus quantitative data from oysters, obtained using the ISO 15216 or equivalent, from oyster beds or retail oysters in the UK and Republic of Ireland. These data were provided by Cefas and the Marine Institute Ireland and were also obtained from relevant literature. The methods for the literature search are provided in Section 8.1, and the data, along with references, are available in Annex I.

Data on GI and GII were quantified separately and combined to enable comparison between baseline and outbreak populations.

It is acknowledged that this baseline data may have come from oysters whose oyster beds produced oysters which caused illness. Therefore, we consider these data to represent the background level of norovirus contamination, which includes oysters capable, and not capable, of causing illness.

Of the 2,924 baseline samples, 1,714 tested positive for GI and 1,546 tested positive for GII – overall 2,029 (69%) samples were positive. 895 (31%) samples did not have detectable norovirus. The maximum recorded value for GI was 21,651 copies/g, and for GII it was 18,024 copies/g, while the combined highest recorded level for both was 24,754 copies/g. The median value of the combined data was 31 copies/g, the arithmetic

mean was 383 copies/g, and the geometric mean was 24 copies/g (for the latter calculation, the zeros were set to 1).

4.2.2 Norovirus in oysters associated with outbreaks

There are few quantitative data on norovirus levels in oysters from batches that have caused disease. Those that exist were identified from the scientific literature (see Section 8.1) and provided by Cefas and the Marine Sciences Institute. The dataset is available as Annex II. These are samples from batches linked to human illness – samples from the same harvesting area but different batches have not been included. Only samples quantified using ISO 15216 or a similar method were included, to ensure data were comparable. We do not know how large the batches of oysters linked to outbreaks are, and what percentage of oysters in that batch caused illness **(uncertainty)**.

Of the 48 outbreak related samples, 35 tested positive for GI and 42 tested positive for GII – overall 46 (96%) samples were positive. One sample (2%) did not have detectable norovirus. The maximum recorded value for GI was 10,500 copies/g, and for GII it was 9,398 copies/g, while the combined highest recorded level for both was 10,500 copies/g. The median value of the combined data was 1,359 copies/g, the arithmetic mean of the data was 2,115 copies/g and the geometric mean of the samples was 874 copies/g (for the latter calculation, the zeros were set to 1).

Half of all outbreak oysters had levels of norovirus higher than 1,300 copies/g.

A two-sample Kolmogorov_Smirnov test showed the baseline and outbreak populations are significantly different ($p \approx 10^{-16}$).

The baseline and outbreak data are shown in Figure 5 as histograms. The proportions of samples that are above the thresholds of 200, 500 and 1,000 copies per gram are different between the baseline and outbreak populations (see Table 6). The outbreak population has a higher proportion of samples with high norovirus levels and the baseline population has a higher proportion of samples with undetectable norovirus.

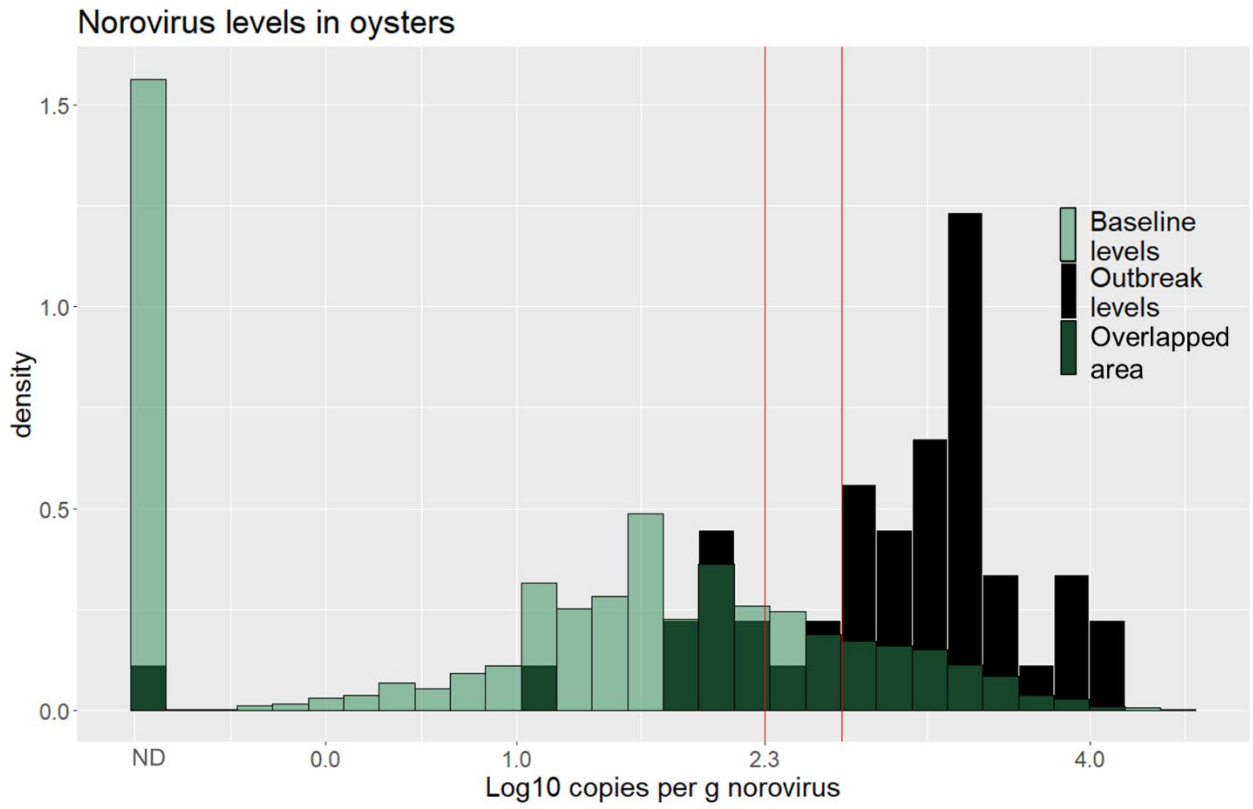

Figure 5: Overlayed density histograms of norovirus levels in log_{10} copies/g of outbreak and baseline data. The levels of norovirus in genome copies/g have been log-transformed. The red lines indicate the log_{10}-transformed values of 200 copies/g and 500 copies/g. A two-sample Kolmogorov Smirnov test showed the baseline and outbreak populations – including those in which norovirus was not detected - are significantly different ($p \approx 10^{-16}$).

Table 6: The number and percentage of results that fall in different quantitative norovirus thresholds within the baseline and outbreak data.

Norovirus levels	Baseline data	Outbreak data
Not detected, ND (%)	887 (29%)	1 (2%)
0.1 to < 200 copies/g (%)	1436 (48%)	9 (19%)
200 to < 500 copies per g (%)	263 (9%)	3 (6%)

500 to < 1,000 copies per g (%)	149 (5%)	7 (15%)
1,000 copies per gram or more (%)	278 (9%)	28 (58%)
All results	3013 (100%)	48 (100%)

4.3 Oyster consumption levels in the UK

Levels of oyster consumption in the UK are low. Of 5094 participants aged 19-64 in the National Diet and Nutrition Survey, 6 reported having eaten oysters within the 4 day window of the survey. Of participants aged 4-18 months in the diet and nutrition survey of infants and young children, 1 out of 2683 had eaten oysters (further information is available in the Appendix, Section 8.2). Scaling up to the UK population of 67 million, that would mean around 4.4 million oyster meals are consumed every year (see the Section 8.2). However, estimates made with a low number of consumers are likely to be inaccurate **(uncertainty)**.

A 2012 report on Pacific Oysters states that around 400 tonnes are produced every year for domestic consumption (Herbert *et al.*, 2012). Assuming 11,110 animals per tonne (Herbert *et al.*, 2012), that results in around 4.4 million Pacific Oysters produced domestically. Native oyster production was estimated to be around 10% or less of Pacific Oyster production.

Of the 6 adults who reported consuming oysters in the National Diet and Nutrition Survey, the mean level of consumption of oyster meat in a meal was 35 g, the 97.5th percentile was 57 g, and the maximum consumption level was 60 g (see Appendix).

A typical serving size in other sources is quoted as being between 1-10 oysters with the average weight of meat from an oyster being 25-30g (Lowther *et al.*, 2010; Hunt *et al.*, 2023). A quantitative exposure assessment model for norovirus in oysters estimated that a serving size of 6 oysters for a test concentration of 200 genome copies/g results in a median value of 919 genome copies ingested (97.5% exposure estimate of 1343 copies). For 6 oysters at a test concentration of 1,000 genome copies/g it estimates a median

exposure of 4,582 genome copies (97.5% exposure estimate of 6,713 copies) (Hunt *et al.*, 2023).

5. Risk characterisation

Of around 400,000 foodborne norovirus cases, an estimated 15,000-30,000 cases are linked to contaminated shellfish each year in the UK. Oysters are a well-known source of norovirus, due to human faecal contamination of oyster beds by wastewater. Through filter feeding, oysters concentrate the virus, which then binds strongly to its digestive tract. As oysters are often consumed raw, they are a high-risk product for norovirus.

Testing options for norovirus are limited and unreliable. There is no assay that is currently able to distinguish between infectious and non-infectious norovirus. PCR-based tests for viral RNA are instead commonly carried out, and these can have low efficiencies and issues with inhibition. In addition, quantifying norovirus levels using PCR can be misleading as the test also detects inactivated particles.

The UK appears to have higher prevalence of norovirus in oysters, and higher levels of baseline norovirus, compared to other countries, likely due to poorer sanitary quality of its waters, particularly in densely populated regions (Food Standards Australia New Zealand, 2017). High levels of norovirus in oysters occur particularly in winter months, due to cold sea temperatures that allow persistence of the virus, and an increase in norovirus amplification in the community. Risk factors for norovirus contamination of oysters are summarised in Cefas, 2015, and these can be valuable indicators for assessing the risk of a batch of oysters.

Control options are limited, as oysters tend to be served raw, and depuration methods at conditions set forth in FSA Scotland (now FSS) and FSA guidance have limited effectiveness at eliminating norovirus. There are data showing that depuration at higher temperatures (15-17ºC) for several days are more effective, and merit consideration. In Ireland, the Food Safety Authority Scientific Committee published advisory guidance recommending that production areas implicated in norovirus outbreaks demonstrate levels of norovirus below 200 copies/g before being able to place their products on the market again (FSAI guidance). In France, risk management actions after an outbreak include oyster bed closures for 28 days and weekly norovirus testing until the production area tests negative. The European Commission has proposed (but not implemented) limits of 200 genome copies/g of digestive tissue in the end-product, or harvest standards

of shellfish collected from the seabed of a maximum of 1,000 copies/g (Hassard *et al.*, 2017); or alternatively, limits of 500 genome copies/g (AAC, 2020). US guidance recommends bed closure for 21 days and states that shellfish associated with norovirus outbreaks could be returned to the harvesting area for at least 60 days (NSSP, 2019).

The infectious dose required to infect 50% of susceptible participants measured in volunteer challenge studies varies from 1,000 to 500,000 genome copies (Teunis *et al.*, 2008, 2020; Leon *et al.*, 2011; Atmar *et al.*, 2014; Rouphael *et al.*, 2022), with modelling studies suggesting it may be even lower, with low dose extrapolation highly uncertain (Van Abel *et al.*, 2017). This variation could be caused by differing infectivity of different norovirus strains or the fact that it is not possible to determine how many of the measured particles are infectious. The large variation means the infectious dose is uncertain, however we can conclude that as the level of genome copies increase, the risk of human norovirus infection increases. It is likely that this is the virus at its most infectious, as it is purified from faecal human samples, whereas the norovirus detected in oysters may be less infectious, having passed through the sewerage system and waterways before being concentrated in the oyster.

Only around 80% of the UK population is estimated to be intrinsically susceptible to norovirus illness, and more may be protected from infection for months or years due to immunity after a previous infection.

Data from batches of oysters linked to outbreaks suggest that 21% of norovirus cases are caused by oysters with levels below 200 copies/g. This is because even low levels of infectious norovirus can cause disease in a significant proportion of susceptible people.

Despite difficulties in quantification and detection of infectious particles, there is a clear link between the number of genome copies in oysters and the ability to cause disease. Oysters from batches linked to outbreaks had significantly higher geometric mean norovirus levels of 874 copies/g, compared to baseline oyster data average of 24 copies/g.

70% of all oysters produced in the UK have detectable norovirus, including a substantial percentage of samples with high norovirus levels, sufficient to cause illness – 9% of generic samples have more than 1,000 copies/g. However, the risk of illness from oysters is much lower – estimated at 1 in 160 oyster meals. This means that quantitative norovirus data alone is not sufficient for determining the risk of illness.

There is **uncertainty** around the actual ingested dose during outbreaks, as the oyster(s) that is consumed and led to disease may have different norovirus levels to the oysters tested from the implicated batch.

Given the uncertainties described, it is not possible to supply a risk level for norovirus PCR test results in isolation. Likelihood of illness for quantitative norovirus levels are supplied in Table 7 and are only applicable in the following scenarios:

- Human wastewater flows into the catchment area of beds (which may be demonstrated by high *E. coli* levels) OR
- An outbreak (i.e., at least two separate[3] cases of disease) epidemiologically linked to oyster bed occurs,

These likelihood values are not applicable to measurements of oyster levels on the market without these (or other) risk factors.

The strong correlation between norovirus levels and colder waters should also be considered, as well as other local risk factors that are known to correlate with norovirus illness or elevated norovirus levels in the water, for instance, heavy rainfall and flooding events.

Table 7: Likelihood of illness and uncertainty values associated with quantifiable norovirus in oysters and other risk factors. *The likelihood of illness when consuming other oysters from that bed is low-medium in instances of test results below 200 norovirus genome copies per gram. This is to recognise that the likelihood may be assessed low in outbreaks with a small number of reported illnesses (or other exceeded risk factors are less extreme) or could be assessed

[3] To ensure they are foodborne, rather than person-person spread.

medium in outbreaks with a large number of reported illnesses (or other exceeded risk factors are more extreme.

Norovirus levels (copies/g)	**Human wastewater contamination from sewerage spills or batch linked to outbreak**	**Likelihood of illness**	**Uncertainty**
1-200	-	**Unknown**	-
1-200	✓	**Low-Medium** (rare, but does occur – occurs regularly) *	High
201-500	-	**Unknown**	-
201-500	✓	**Medium** (occurs regularly)	High
501-1,000	-	**Unknown**	-
501-1,000	✓	**High** (occurs very often)	High
>1,000	-	**Unknown**	-
>1,000	✓	**Very high** (events occur almost certainly)	High

Noroviruses are very infectious RNA viruses that predominantly spread from person-to-person but can also be transmitted through food. Illness is usually mild, and symptoms clear after a day or a few days in most people.

For this reason, the severity of detriment to the UK population is **low** (mild illness: not usually life-threatening, usually no sequelae, normally of short duration, symptoms are self-limiting e.g., transient diarrhoea), and there is **low** uncertainty associated with this (there are solid and complete data available; strong evidence is provided in multiple references; authors report similar conclusions).

Tables from ACMSF (ACM/1334) adapted from EFSA 2006 modified from OIE 2004 are provided below, describing the qualitative categories for the risk characterisation (Table 8, Table 9, Table 10).

The current FSA and FSS positions are that testing of oysters from batches that are epidemiologically linked to outbreaks where cases experience symptoms typical of norovirus cannot determine infectivity. The lack of an infectious assay, the variability in test results, the typically poor efficiency of extraction and the cost are balanced against any additional information the results may provide on the source of the outbreak. The epidemiological evidence is considered sufficient to declare a norovirus outbreak.

However, there is merit in using norovirus testing within the information contextualised as above, for instance as a preventative tool in the case of adverse weather conditions that may lead to contamination of the oyster beds or to determine the effectiveness of interventions.

Table 8: definition of qualitative categories for probability of occurrence

Frequency category	**Interpretation**
Negligible	So rare that it does not merit to be considered
Very Low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often
Very High	Events occur almost certainly

Table 9: definitions of qualitative categories for severity of consequence

Severity category	Interpretation
Negligible	No effects, or so mild they do not merit to be considered
Low	Mild illness: not usually life-threatening, usually no sequelae, normally of short duration, symptoms are self-limiting (e.g., transient diarrhoea)
Medium	Moderate illness: incapacitating but not usually life-threatening, sequelae rare, moderate duration (e.g., diarrhoea requiring hospitalisation)
High	Severe illness: causing life-threatening or substantial sequelae or illness of long duration (e.g., chronic hepatitis)

Table 10: definitions of qualitative categories for expressing uncertainty

Uncertainty category	Interpretation
Low	There are solid and complete data available; strong evidence is provided in multiple references; authors report similar conclusions
Medium	There are some but no complete data available; evidence is provided in small number of references; authors report conclusions that vary from one another
High	There are scarce or no data; evidence is not provided in references but rather in unpublished

Uncertainty category	Interpretation
	reports or based on observations, or personal communication; authors report conclusions that vary considerably between them

6. Uncertainties and Evidence gaps

Key uncertainties contributing to the assignation of the uncertainty to the public health risk associated with norovirus quantitative data derived solely from ISO 15216:

- Norovirus quantification using ISO 15216 quantifies the total norovirus copy number within oysters, and not necessarily the quantity of intact norovirus which are capable of causing infection. This means that theoretically, an oyster with a high number of norovirus may not contain any norovirus particles capable of causing illness, or oysters with low numbers may contain infectious particles.
- The samples of oysters from batches associated with norovirus cases are limited (48 samples only). There is uncertainty about the levels required to cause illness.
- The levels of norovirus in the oysters that are consumed and cause illness and how well they correlate to the norovirus levels of other oysters in that batch (that are quantified) are unknown.
- The size of batches linked to outbreaks is unknown – therefore the proportion of oysters linked to illnesses in a batch with measured norovirus level is unknown.
- Baseline oyster data are not ‘non-outbreak’ oyster data. Baseline data should be interpreted as the overall background levels of norovirus contamination in UK/Irish oysters, and there is uncertainty about the proportion of baseline oysters which are capable of causing illness.
- Underreporting ratio for norovirus illness caused by oysters. The current estimate of the overall underreporting ratio for the UK population infected with norovirus per year has been provided. However, the exact underreporting ratio of norovirus illness caused by oysters is not known and would be useful in more accurately estimating the health burden attributed to norovirus in raw or less than thoroughly cooked oysters.

- ISO 15216 has permitted tolerances of extraction efficiency and PCR inhibition, as well as intra- and inter-laboratory variation, which creates uncertainties when interpreting individual test results. This stochastic behaviour is especially prominent when testing oysters with low levels of norovirus RNA.
- Frequency of norovirus misdiagnosis of cases that are caused by other enteric viruses such as rotavirus, astrovirus, etc. Unless confirmed with sequencing in a laboratory, individuals infected with norovirus may be misdiagnosed with a different enteric virus, or vice versa.

We suggest the following to fill in the most important evidence gaps:

- The most useful development would be a norovirus infectivity assay to measure viable norovirus particles. However, there are significant barriers to this (Manuel, Moore and Jaykus, 2018).
- The uncertainty around the levels of norovirus in oysters capable of causing disease could be reduced with further sampling. If 144 more samples were taken, this would approximately double the precision with which we could discriminate between groups (FSA Statistics team, 2023). This could be done in conjunction with collecting metadata such as: the number of illnesses, the total batch sizes, the class of implicated beds etc. from UK outbreaks.

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8. Appendix

8.1 Literature search

A literature search was performed to obtain data on:

- Quantitative norovirus levels in oyster batches linked to outbreaks.
- Quantitative norovirus levels in oysters at producers or retail in the UK or Republic of Ireland

This review specifically considers data related to norovirus levels in the Pacific oyster (*Crassostrea gigas*) and the European native oyster (*Ostrea edulis*) in the UK.

In brief, 954 papers were returned from the databases and were screened for relevance.

5 papers remained (alongside 14 other papers excluded because they contained duplicates of the datasets provided directly by Cefas and Marine Institute) after screening.

8.1.1 Overview

Although not a formal systematic review, the principles of the PRISMA systematic review methodology were applied to the literature search (Moher *et al.*, 2009). This involved the following steps:

- defining review questions and developing the eligibility criteria
- literature searches
- screening studies for inclusion or exclusion
- data collation
- data presentation
- interpretation and conclusions

8.1.2 Review questions

The first stage in the literature review process involved definition and analysis of the review question(s) to identify the key elements and clarify the scope. The Scope asks for a Risk Assessment to support development of advice and guidance to manage outbreaks of norovirus in oysters. The review questions were therefore defined as follows:

- What is the public health risk associated with consumption of raw oysters with a range of norovirus RNA levels?
- How do results below the limit of detection or quantification (LOD/LOQ) and inter-laboratory differences in LOD/LOQ affect the interpretation of results and the risk level?

8.1.3 Database searches

Three databases were searched to retrieve relevant literature. These were Scopus, PubMed, and Google Scholar. A search was also conducted on Google to return news articles, papers, and websites of relevance (including guidance from competent authorities). Following manual screening, results were imported directly into the reference management software (Zotero 5.0.82, https://www.zotero.org/). Searches were conducted looking for keywords in the title and abstract.

8.1.4 Search strategy

The search string used for PubMed and Scopus is shown below.

TITLE-ABS-KEY (norovirus OR (norwalk AND virus)) AND TITLE-ABS-KEY (oyster* OR (crassostrea AND gigas) OR (ostrea AND edulis))

The search string in Google and Google Scholar was:

"norovirus" OR "norwalk*" AND "oyster*" OR "Crassostrea gigas" OR "Ostrea edulis"

The searches on Google and Google Scholar were conducted on 14/11/22 and were completed as a supplementary resource to the systematic searches on PubMed and Scopus, the search was conducted on the first 5 pages of results. An additional search was conducted on Google Scholar with the same search string as previous but including AND "outbreak" AND "PCR" OR "RT-PCR" AND "United Kingdom" OR "UK" on the 18/11/22.

The results from these databases were combined with a total of 954 returned. Duplicates were removed in Excel.

8.1.5 Manual screening

After removal of duplicates, 540 papers remained, which were then manually screened by abstract to determine suitability for inclusion. This process was performed

independently by three FSA/FSS researchers in line with good practice guidance for systematic literature reviews. Papers were screened out using the criteria listed in Table 11 based on reviewer interpretation. In the case of disagreements, papers were discussed until a consensus was achieved, with the default of continuing to include the paper in the next stage of the process.

Table 11: Categories for exclusion and inclusion from manual sifting

To Exclude	To include
E. coli or other proxies or organisms Modelling studies Method development/ methods other than qPCR Impact of depuration/cooking/other methods on norovirus Norovirus levels in oysters outside of UK/EU Genome/molecular studies Reviews Foods other than oysters	Quantitative levels of norovirus in UK/Republic of Ireland oysters Infectious dose in outbreaks/illness worldwide

After the abstract screen, there were 115 papers remaining. Date limitation was then applied to include only papers published from 2006 onwards. Following the date limitation, 68 papers remained.

These papers were then read fully and 19 were included based on their methodology (see Table 12).

Table 12: Categories for inclusion and exclusion based on methodology.

To include	To exclude
Quantitative data following ISO 15216 Quantitative data following method comparable to ISO 15216	Other methods Qualitative or semi-quantitative results

49 papers were excluded as 1) the samples were not representative of oysters on the market or, 2) the data was non-UK/Republic of Ireland baseline data or, 3) raw data was not provided. Additionally, 14 papers were identified as duplicates as the data from those papers had already been provided by Cefas (UK), or the Marine Institute (Republic of Ireland).

8.1.6 Data collation

Following manual screening, 5 papers remained (alongside the Cefas and Marine Institute datasets). The quantitative data on levels of norovirus (G1 and GII) copies/g were extracted and collated using a standardised system independently by two FSA/FSS researchers and are available in Annex 1 (see also Table 13).

Table 13: Information extracted from research papers during the literature search.

Data category	Information to be extracted
Methodology	Is the method ISO 15216? Is the method comparable to ISO 15216? What is the LOD/ LOQ? Country

Data category	Information to be extracted
Q1 - Quantitative data on outbreaks associated with Norovirus and oysters (including levels below LOD/LOQ)	Does the paper have quantitative data on oysters associated with outbreaks?
Q2 - Quantitative data on Norovirus in oysters (control)	Does the paper have quantitative data on baseline norovirus levels in oysters?
Q3 - Inter-laboratory differences in detecting norovirus, particularly LOD and LOQ	LOD LOQ Method efficiency

8.1.7 Data clean-up

Sample results classed as 'not detected' were assigned a value of 1 when calculating the geometric mean, 0 otherwise. Samples with results that were detectable but unquantifiable were assigned a value of LOQ/2. Three outbreak samples only quantified GII, due to epidemiological findings which indicated that the causative agent of infection was norovirus belonging to GII (Le Guyader *et al.*, 2010; Loury *et al.*, 2015). GI levels in this case were set to 0 (this could potentially underestimate the level of GI). Some data were excluded from (Hunt *et al.*, 2020) because one site where oysters were collected was an unclassified site with high levels of sewage outflow and were not meant to represent oysters which could be reasonably sold for raw consumption. The other site was a class A site, and therefore oyster data from this site were retained.

8.2 Oyster consumption data

Consumption data for oysters in the UK were obtained from the National Diet and Nutrition Survey (Bates *et al.*, 2014, 2016, 2020; Roberts *et al.*, 2018), and the diet and nutrition survey of infants and young children (Department of Health, 2013). These are presented in Table 14.

Acute consumption, or Acute Maximum Day consumption, is the maximum amount of a particular food that is eaten in a day over any of the 4 survey days. The amount of single eating events of a food are added per day and the maximum over the survey days will give the Acute Maximum Day consumption. Chronic consumption is the average consumption per day over the survey period. For the purposes of this risk assessment, acute consumption figures were used.

Table 14: Consumption of oysters in the UK by age group, from the National Diet and Nutrition Survey, and the diet and nutrition survey of infants and young children. This does not include oysters consumed as part of a recipe. Consumption is given in grams per person per day

Age	Number of consumers	Chronic consumption Mean	Chronic consumption 97.5th Percentile	Chronic consumption Maximum	Acute consumption Mean	Acute consumption 97.5th Percentile	Acute consumption Maximum	Number of respondents in Population group
4-18 months	1	1.7	1.7	1.7	6.7	6.7	6.7	2683
1.5 - 3 yrs	0	0	0	0	0	0	0	1157
4 - 10 yrs	0	0	0	0	0	0	0	2537
11 - 18 yrs	0	0	0	0	0	0	0	2657
19 - 64 yrs	6	9.2	14	15	35	57	60	5094
65 + yrs	0	0	0	0	0	0	0	1538

The Office for National Statistics' Mid-Year Population Estimates, UK, June 2021 (see Table 15) were used together with the NDNS consumption data to estimate the yearly oyster meals in the UK (Table 16).

Table 15: UK population by age group, based on Office for National Statistics' Mid-Year Population Estimates, UK, June 2021.

Age Groups	Population
0 to 4	3,580,269
5 to 9	3,933,947
10 to 14	4,034,833
15 to 19	3,794,214
20 to 24	4,034,799
25 to 29	4,367,126
30 to 34	4,655,236
35 to 39	4,477,368
40 to 44	4,226,466
45 to 49	4,214,569
50 to 54	4,640,482
55 to 59	4,573,856
60 to 64	3,956,096
65 to 69	3,354,034
70 to 74	3,345,116
75 to 79	2,490,287
80 to 84	1,698,442
85 to 89	1,050,295
90 and over	598,857

Table 16: Calculated estimates of the yearly oyster meals for the UK population aged 18 and under, and aged 19 and above. Total meals for all age groups is 4,421,633.

-	0 to 18 years	19 + years
Consumers	1	6
Number of respondents	9034	6632
Annual consumption rate	0.010100731	0.082554282
Population	15,343,263	51,683,029
Annual meals	154,978	4,266,655

8.3 Dose response data from challenge studies in human volunteers

Teunis et al., 2008

Human volunteer trials carried out by Teunis *et al.*, (2008), using serial dilutions of norovirus, found a dose-dependent probability of illness in secretors (susceptible individuals exhibiting the histo-blood group antigen) ranging from 11% (1/9 illness in participants) at a dose of 10^3 norovirus genome copies to 67% (4/6 illness in participants) at a dose of 10^8 norovirus genome copies (see Table 17). Non-secretors, less-susceptible individuals, did not become ill at any dose. In this experiment the dose response assessment was restricted to secretors.

Table 17: Effects of norovirus challenge study in human volunteers, with norovirus 8fIIa inoculum, as reported by Teunis *et al.*, (2008).

Dose (genome copies)	Illness in participants
3.2×10^1	0/8 (0%)
3.2×10^2	0/9 (0%)
3.2×10^3	1/9 (11%)
3.2×10^4	1/3 (33%)
3.2×10^5	6/8 (75%)
3.2×10^6	1/7 (14%)
3.2×10^7	2/3 (67%)
3.2×10^8	4/6 (67%)

The same study challenged another 27 secretor-positive participants with a different norovirus inoculum, with results presented in Table 18. The 8fIIa inoculum had been stored as a stock for 25 years, with the solution containing aggregated particles. A stool sample from a participant infected with 8IIfa inoculum was used to prepare 8IIfb inoculum, which contained dissociated viral particles.

Table 18: Effects of norovirus challenge study in human volunteers, with norovirus 8fIIb inoculum, as reported by Teunis *et al.*, (2008).

Dose (genome copies)	Illness in participants
6.9×10^5	2/8 (25%)
6.9×10^6	7/18 (39%)
2.1×10^7	NA/1

Leon *et al.*, 2011

Challenge with 10^4 norovirus genome copies in oysters resulted in infection in 7 out of 15 subjects (47%) (Leon *et al.*, 2011). The individuals in the study were genetically susceptible to norovirus and were asked to consume sodium bicarbonate prior to the challenge to reduce their stomach acidity.

Frenck *et al.*, 2012

In a challenge study 13/23 secretors (57%) became ill when they were given 5 x 10^4 genome copies (Frenck *et al.*, 2012). 16/23 of participants were infected, although only 13 showed symptoms. Of the non-secretors, 1/17 (6%) became ill.

Atmar *et al.*, 2014

Atmar *et al.*, 2014, estimated that a level of 2800 genome copies is necessary for infecting 50% of general secretors, with those of blood group O or A even more susceptible. They challenged secretors with a range of doses (see Table 19).

Table 19: Effects of norovirus challenge study in human volunteers, as reported by Atmar *et al.*, 2014.

Dose (genome copies)	Infection in participants
1.9 x 10^2	1/13 (8%)
1.9 x 10^3	7/13 (54%)
1.9 x 10^4	7/8 (88%)
1.9 x 10^6	6/7 (86%)

Rouphael *et al.*, 2022

A recent dose-response study with norovirus GII.2 estimated the infectious dose required to infect 50% of secretor-positive participants as 5.1 x 10^5 genome copies, which is ten to one hundred-fold higher than previous studies (Rouphael *et al.*, 2022). This may be due to different statistical approaches for calculating the value, or differences in the norovirus strains used. Data are presented in Table 20. 50% of secretor-negative participants became ill when ingesting the highest dose of 1.2 x 10^7 genome copies.

Table 20: Effects of norovirus challenge study in human volunteers, as reported by Rouphael *et al.*, 2022.

Dose (genome equivalent copies)	**Illness in participants**
1.2 x 10^4	1/9 (11%)
1.2 x 10^6	3/8 (38%)
1.2 x 10^7	10/12 (83%)

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