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RISK PROFILE:
NOROVIRUS
IN MOLLUSCA (RAW)

Prepared as part of a New Zealand Food Safety Authority
contract for scientific services

by

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RISK PROFILE:
NOROVIRUSES
IN MOLLUSCA (RAW)

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EXECUTIVE SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (http://www.nzfsa.govt.nz/about-us/risk-management-framework/index.htm) approach taken by the New Zealand Food Safety Authority (NZFSA).

The food/hazard combination addressed by this Risk Profile is norovirus in mollusca (raw).

The NZFSA have commissioned this Risk Profile in order to re-evaluate the risk in the light of recently published information collected from multi-site shellfish quality surveys in New Zealand and to address the following specific risk management question:

- Has the risk of human infection from norovirus in mollusca (raw) changed since the previous Risk Profile (Greening *et al.*, 2003a)?

Human noroviruses are now the most common cause of outbreaks of epidemic non-bacterial gastroenteritis world-wide (Siebenga *et al.*, 2009). Previously known as Norwalk-like viruses (NLVs) and small round structured viruses (SRSVs), these viruses belong to the *Caliciviridae* family and are 26-35 nm non-enveloped single stranded positive-strand RNA viruses.

Human noroviruses contaminate filter feeding bivalve molluscan shellfish through faecal contamination of growing waters.

The phylum Mollusca includes easily recognised bivalve molluscs, as well as the cephalopods such as squid and octopus (New Zealand Fishing Industry Board 1981). For this Risk Profile, the relevant species are marine bivalve molluscs which are filter feeders, and thus able to accumulate pathogenic microorganisms. These include: clams (including cockle, pipi, toheroa, tuatua), mussels (blue, green, horse), oysters (dredge, Pacific, rock), and scallops.

All these mollusca occur as feral (i.e. naturally occurring, not farmed) populations around New Zealand. Large quantities of Pacific oysters and green mussels are farmed commercially as aquaculture and dominate the mollusca consumption in New Zealand. Imported shellfish represent a minor part of the food supply.

Raw molluscan shellfish are an infrequently consumed food in New Zealand, when considered as part of the national consumption overview. However, consumption of recreationally gathered shellfish is likely to be concentrated in certain regional and ethnic populations, and so exposure will occur mostly in those populations.

There is accumulating data to indicate frequent contamination of New Zealand shellfish (both commercial and feral) with norovirus. Even low levels of contamination will present a high probability of infection.

From 1994 to 2008 outbreaks of norovirus infection have been identified where the vehicle has been oysters, either from commercial production in New Zealand, or imported.

In terms of volume, commercially grown mussels and oysters are the largest segment of the molluscan food supply in New Zealand. Of the feral populations, the commercial cockle harvest appears to be the most important. The volume of imported molluscs is very small in comparison with the domestic supply, but imported oysters have caused norovirus outbreaks in New Zealand.

Based on the expert elicitation attribution estimates there may be 16% (approximately 65,000 cases) of norovirus infections transmitted by shellfish each year. However, this estimate has high uncertainty, as the estimates of both attribution and total number of cases, have wide confidence intervals.

The regular identification of outbreaks of infection linked to contaminated oysters over the past 15 years indicates an ongoing risk, and raw oysters are the most commonly identified vehicle. Mussels and scallops have not been identified as causing outbreaks; these types of molluscs are probably of lower risk of contamination due to their occurrence in deeper water (while feral mussels can occur in the intertidal zone, aquaculture mussels are grown in deeper water). Cockles have not been identified as the cause of a norovirus outbreak in New Zealand but shellfish monitoring programmes have found contamination in this type of shellfish.

It is unclear whether the risk of norovirus infection from commercial shellfish for the New Zealand population has changed since the previous Risk Profile was completed in 2003. However, the risk has been better characterised as a result of surveys including the multi-site and Tauranga Harbour surveys, and evidence for widespread norovirus contamination of shellfish, particularly feral shellfish, has been obtained.

Commercial oyster related outbreaks of norovirus infection continue to be identified, particularly in the Auckland region. Although widespread contamination of feral shellfish with norovirus has been demonstrated, surveillance data linking this contamination with human illness have not yet been reported.

Recent improvements in detection methods have facilitated the collection of data to better describe and manage this risk. Reducing sources of human faecal contamination in the Bay of Islands area have demonstrated how the risk can be better managed.

This Risk Profile has identified a number of data gaps which if filled would contribute to the increasing knowledge on noroviruses in foodborne disease, especially BMS in New Zealand. These data gaps are in the areas of surveillance, exposure assessment, detection methods, effectiveness of methods for virus removal and/or inactivation, virus recombination and the zoonotic potential of noroviruses. Many of the data gaps cannot be addressed until there is a robust method to assess and quantify the infectivity of human norovirus. This is still not possible.

1   STATEMENT OF PURPOSE

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles are part of the Risk Management Framework (http://www.nzfsa.govt.nz/about-us/risk-management-framework/index.htm) approach taken by the New Zealand Food Safety Authority (NZFSA). The Framework consists of a four step process, as shown in Figure 1.
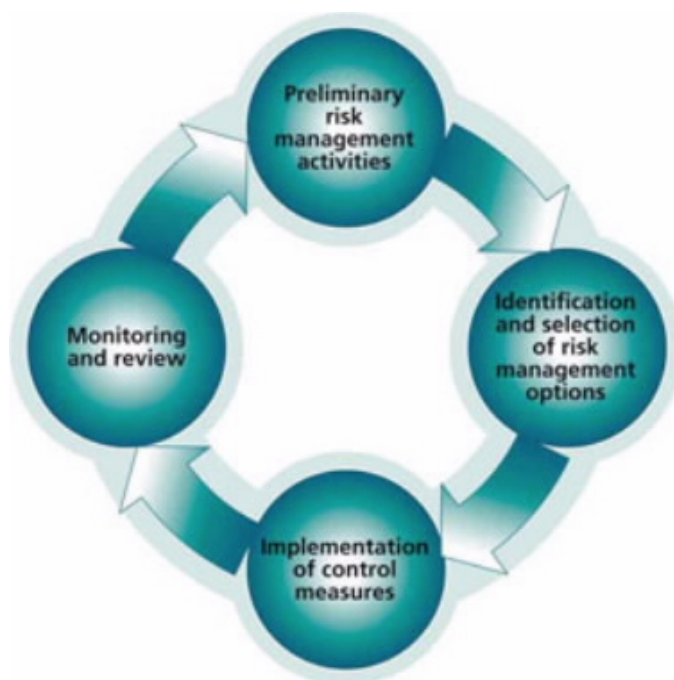
Figure 1: The four steps of the Risk Management Framework

This initial step in the RMF, Preliminary Risk Management Activities, includes a number of tasks:
- identification of food safety issues
- risk profiling
- establishing broad risk management goals
- deciding on the need for a risk assessment
- if needed, setting risk assessment policy and commissioning of the risk assessment
- considering the results of the risk assessment
- ranking and prioritisation of the food safety issue for risk management action.

Risk profiling may be used directly by risk managers to guide identification and selection of risk management options, for example where:

- rapid action is needed
- there is sufficient scientific information for action
- embarking on a risk assessment is impractical.

1.1 Food/hazard Combination and Risk Management Questions

The food/hazard combination addressed by this Risk Profile is norovirus in mollusca (raw).

The NZFSA have commissioned this Risk Profile in order to re-evaluate the risk in the light of recently published information collected from multi-site shellfish quality surveys in New Zealand and to address the following specific risk management question:

- Has the risk of human infection from norovirus in mollusca (raw) changed since the previous Risk Profile (Greening *et al.*, 2003a)?

2   HAZARD AND FOOD

2.1   The Hazard: Noroviruses

Human noroviruses are now the most common cause of outbreaks of epidemic non-bacterial gastroenteritis world-wide (Siebenga *et al.*, 2009). Previously known as Norwalk-like viruses (NLVs) and small round structured viruses (SRSVs), these viruses belong to the *Caliciviridae* family and are 26-35 nm non-enveloped single stranded positive-strand RNA viruses.

The only known reservoir for human norovirus is human faeces. Human noroviruses contaminate filter feeding bivalve molluscan shellfish through faecal contamination of growing waters. Fresh produce may be contaminated by poor hygiene practices by food harvesters (contaminated irrigation or processing water). Manually prepared ready-to-eat foods may be contaminated by infected food processors and handlers.

In addition to contaminated food or water, person-to-person transmission is important, either directly or via contaminated surfaces and objects. In outbreaks, multiple transmission routes may occur simultaneously.

2.1.1   Types causing disease

Noroviruses are divided into 5 genogroups, GI-V, with characteristics shown in Table 1. Twenty five different genotypes (also known as strains) that cause disease in humans are now recognised across the relevant genogroups.

Table 1: Norovirus genogroups (Koopmans, 2008).

Norovirus genogroup	Host	Identified from human cases
I	Human	Frequently
II	Human	Frequently
III	Animal	N/A
IV	Human	Occasionally
V	Animal	N/A

Between 1995 – 2008, a number of new recombinant strains and also several GII.4 variants have emerged (Bull *et al.*, 2007; Koopmans, 2008). GII.4 strains are most often reported in outbreaks from healthcare settings, and the appearance of these GII.4 variants has coincided with major peaks in outbreak reporting (Siebenga *et al.*, 2009).

Multiple norovirus strains are frequently identified in shellfish and in human faecal specimens from cases associated with gastroenteritis outbreaks following consumption of contaminated shellfish (Costantini *et al.*, 2006; Gallimore *et al.*, 2005; Le Guyader *et al.*, 2006b; Le Guyader *et al.*, 2008). The presence of multiple norovirus strains is now believed to facilitate norovirus recombination within the human gut with the subsequent emergence of novel norovirus recombinants.

The occurrence of both human and animal caliciviruses in shellfish has been reported (Le Guyader *et al.*, 2008), presumably resulting from simultaneous contamination of water by human and animal species. In a study of shellfish in US coastal waters, a range of animal and

human enteric caliciviruses in shellfish, including human, porcine and bovine noroviruses were identified (Costantini *et al.*, 2006) and several shellfish samples contained both animal and human noroviruses.

2.1.2   Cross-species infection

While viruses are usually host specific, there have been occasional research reports that suggest cross-species infection (or carriage) of human noroviruses in animals, and hence the potential for zoonotic transmission (Koopmans, 2008).

Faecal samples from pigs, beef and dairy cattle, as well as retail meat samples were tested for norovirus in Canada (Mattison *et al.,* 2007). The specific norovirus strains present were identified by sequence analysis. Swine and bovine norovirus strains were detected in some of the faecal samples, as expected, but human norovirus was also found in swine and dairy cattle faecal samples. Furthermore, human norovirus was found in one retail sample of raw pork.

A gnotobiotic[1] calf was experimentally infected with a human GII norovirus strain, which may offer a future animal model for research (Souza *et al.,* 2008). Distinct GII strains (which normally infect only humans) have been identified in pigs and recently the first identification of GIV noroviruses in animals was reported (Martella *et al.*, 2007).

Bovine, ovine and porcine noroviruses have been identified in faecal samples from those animals in New Zealand (Wolf *et al.*, 2009) but there are no reports of cross-species transmission to humans.

While these reports are suggestive, zoonotic transmission of norovirus is not currently considered a significant pathway.

2.1.3   Detection Methods

Norovirus identification was difficult prior to development of molecular methods because human noroviruses are not culturable, and their wide genetic diversity limits the use of traditional immunology and serotyping assays. There are no animal models for human norovirus strains.

The introduction of reverse transcriptase polymerase chain reaction methods (RT-PCR) and nucleotide sequencing methods over the last few years has provided methods for direct detection and classification of these viruses in faecal specimens and in environmental samples, including shellfish. In recent years, real-time RT-PCR methods have been introduced which provide both confirmation and semi-quantitation in a single assay.

Genotype is routinely determined for noroviruses identified from outbreaks in New Zealand by sequencing part of the genome. Overseas databases are used to provide reference sequences, and New Zealand data are contributed to international networks.

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[1] ∗ Gnotobiotic animals (also 'gnotobiote' or 'gnotobiont') are born in aseptic conditions, removed from the mother by Caesarean section. They are reared in the laboratory, exposed only to those microorganisms that the researchers wish to be present in the animal. They are used in research into the symbiotic relationship between an animal and the microorganisms that inhabit its body.

The quantification of noroviruses is performed by determination of the amount of extracted viral RNA. The amount of RNA is determined by using real time RT-PCR and expressed as RT-PCR copies per gram. An RNA copy is assumed to be equivalent to a viral particle. Experiments to determine recovery of viral RNA during extraction from shellfish tissue have shown that recovery is highly variable (20 – 100%), and so quantification measurements will almost always be an underestimate (Hewitt and Greening, 2009).

Supplementary information on norovirus characteristics and detection is provided in Appendix 1.

2.2   The Food: Mollusca (raw)

Although this Risk Profile includes some information on cooking mollusca, the risk being considered is from consumption of raw mollusca.

The phylum Mollusca includes easily recognised bivalve molluscs, as well as the cephalopods such as squid and octopus (New Zealand Fishing Industry Board 1981). For this Risk Profile, the relevant species are marine bivalve molluscs which are filter feeders, and thus able to accumulate pathogenic microorganisms. These include:

- Clams, including:
  - Cockle (*Austrovenus stutchburyi)* (also known as littleneck clam)
  - Pipi *(Paphies australis*)
  - Toheroa (*Paphies ventricosa*)
  - Tuatua (*Paphies subtriangulata* and *Paphies donacina* (deepwater))
- Mussel, blue (*Mytilus edulis aoteanus*) (also known as rock mussel)
- Mussel, green *(Perna canaliculus*) (also known as green lipped mussel)
- Mussel, horse (*Atrina pectinata* and *Atrina zelandica*)
- Oyster, dredge (*Ostrea chilensis*)
- Oyster, Pacific (*Crassotrea gigas*)
- Oyster, rock (*Saccostrea glomerata*)
- Scallop (*Pecten novaezelandiae*)

All these mollusca occur as feral (i.e. naturally occurring, not farmed) populations around New Zealand.

The Quota Management System operated by the Ministry of Fisheries (http://fs.fish.govt.nz/Page.aspx?pk=81&tk=248) controls the amounts (Total Allowable Catch (TAC)) of some feral mollusca populations that can be harvested commercially, although in fisheries where non-commercial users are involved (e.g. customary Maori or recreational fishers), a quantity of stock is set aside for them before the commercial catch (TACC) is set. Details of quota managed species and allocated amounts for 2007-2008 are given in Table 2. The amounts listed represent a summation of data for specific areas around New Zealand.

TACC allocations may vary from year to year. According to the Seafood Industry Council website (http://www.seafoodindustry.co.nz/species accessed 1 Sept 2009) in 2008-2009 the TACC were as listed in Table 2, except for dredge oysters (2,100 tonnes), and commercial fishing of green mussels is no longer conducted.

The principal feral mollusca gathered by commercial operations are cockles, while those with the greatest amounts set aside for customary and recreational gathering are for cockles, pipis, green mussels, and tuatua.

Note that the customary and recreational amounts in Table 2 represent amounts considered as allocations for management of the fisheries; they do not represent measurement of actual amounts harvested.

Table 2: Quota Management Amounts for relevant feral mollusca for the 12 months to September 2008 (from: http://fs.fish.govt.nz/Page.aspx?pk=16&tk=114 accessed 1 Sept 2009)

Name	Species	Reported catch (tonnes)	TACC* (tonnes)	Customary (tonnes)	Recreational (tonnes)
Cockle	*Austrovenus stutchburyi*	1148	3214	161	221
Pipi	*Paphies australis*	144	204	242	242
Green mussel	*Perna canaliculus*	152	1720	467	315
Scallop	*Pecten novaezelandiae*	226	841	79	79
Dredge oyster	*Ostrea chilensis*	62	573	12	12
Deep water tuatua	*Paphies donacina*	3	118	67	67
Tuatua	*Paphies subtriangulata*	0	43	137	137
Horse mussel	*Atrina zelandica*	0.5	29	9	9

* Total Allowable Commercial Catch

The Ministry of Fisheries sets limits on the number and size of the quota managed mollusca that can be gathered non-commercially by individuals under customary or recreational allocations: (see: http://www.fish.govt.nz/en-nz/Recreational/default.htm?WBCMODE=PresentationUnpublished).

Pacific oysters and green mussels are farmed commercially as aquaculture in New Zealand. Most New Zealand Pacific oysters are rack-farmed in the inter-tidal zone close to the coast. Mussels are grown commercially on ropes in deep water. According to data from Aquaculture New Zealand 90,085 tonnes of greenshell mussels and 2,852 tonnes of Pacific oysters were harvested in 2008 (Source: Aquaculture New Zealand levy data). With limited robust domestic sales information available, it is assumed that approximately 30% of mussels and 40% of oysters are consumed domestically. This means that oysters and mussels from aquaculture dominate mollusca consumption in New Zealand.

For risk management of hazards in mollusca the New Zealand Food Safety Authority uses the designation Bivalve Molluscan Shellfish (BMS), for example in controls on imports.

In this Risk Profile the terms "mollusca" and "shellfish" are interchangeable.

2.2.1   Imported shellfish

New Zealand imports modest quantities of shellfish and shellfish meat, import volumes being influenced by market dynamics such as exchange rate changes etc. Data obtained from Statistics New Zealand show that in the year to September 2007 approximately 54 tonnes of oysters and 490 tonnes of scallops were imported. These amounts increased significantly in the year ending September 2008, with approximately 70 tonnes of oysters and 820 tonnes of scallops being imported. The majority of these imports were frozen, and largest single source was the Peoples Republic of China. China, followed by Japan, remains the major source of imported scallops.

There has been considerable volatility in sources for oyster importation. In 2001, 80 tonnes were imported from South Korea. This quantity increased to 160 tonnes in 2005. This reflects changes made to the import standards and requirements for bivalve molluscan shellfish described below.

In 2004 NZFSA reviewed the risk management approach applied to imports of prescribed or high risk foods. To ensure high risk foods are safe for human consumption a decision was made to move away from a stand alone sampling and testing at the border to a programme that recognises exporting country food safety controls with agreed assurances (certificates) provided.

In 2006 NZFSA revised the standard and import requirements applying to imported bivalve molluscan shellfish. Following a period of consultation, the Food (Prescribed Foods) Standard was amended and the associated Imported Food Requirement updated to better manage the risks associated with these shellfish. NZFSA requested that exporting countries demonstrate that BMS is derived from a regulated environment that manages hazards and meets New Zealand's requirements. This could be demonstrated by replicating standards of the New Zealand shellfish programme, or have their production systems determined as equivalent to New Zealand, EU or US programmes, which are already recognised by NZFSA as meeting New Zealand public health outcomes.

One of the drivers for reviewing the import requirements for bivalve molluscan shellfish was the outbreaks of norovirus which investigations linked to consumption of South Korean raw oysters that were labelled as being required to be cooked prior to consumption. South Korea, like all other exporting countries was asked to demonstrate that their programmes for managing the hazards associated with bivalve molluscan shellfish comply with or are equivalent to the New Zealand programme.

In 2008 limited trade in Korean oysters continued on a case-by-case basis with allowance made for processing and re-export of product where appropriate certification could be provided that products was suitable for direct export to the European Union, with 46 tonnes imported from that source.

It is difficult to determine what proportion of shellfish consumption by New Zealanders these amounts represent, as there are no details on whether the weights include shells or just flesh. Further it is not clear from statistical data whether these products are imported to be processed or sold in New Zealand for human consumption or re-exported.

2.2.2   Exported shellfish

With exports in the region of $NZ1.36 billion in 2008, the seafood sector ranks amongst the top five export sectors in the New Zealand economy, and Australia, Hong Kong and the USA are the largest markets. In 2008 by far the largest component of the shellfish export market was mussels, of which 32,038 tonnes were exported, worth $187 million. Other relevant shellfish exports were: oysters 1,869 tonnes (worth $17 million); and "other" shellfish (excluding squid) 1,053 tonnes (worth $8 million). Although exported volumes decreased from 2007 to 2008, the value of shellfish exports increased overall.

Note that the forms in which mussels and oysters are exported are different to the whole mussel harvest data quoted in Section 2.2; therefore the weights quoted are not comparable.

(Information from the New Zealand Seafood Industry website: http://www.seafood.co.nz/factfile accessed 21 August 2009)

2.3   Sources of contamination of the food by the hazard

The only known reservoir for human norovirus is human faeces.

Faeces from infected humans may contaminate soil or water. Faecal pollution from sewage discharges, septic tank leachates and boat discharges has caused contamination of shellfish beds, recreational water, irrigation water and drinking water. Norovirus are believed to survive for long periods in the environment and have been detected in shellfish 8-10 weeks after contamination (Greening *et al.,* 2003).

Bivalve molluscs feed and respire by inducing a current of water to flow over a series of complex gill structures that capture suspended particulate matter, passing it towards the mouth where it may be ingested or rejected as pseudo–faeces. Oysters can filter 10-20 litres of water per hour. These shellfish are capable of concentrating viruses that may be present in water, resulting in viral concentrations far exceeding those of the surrounding water (Grohmann, 1997; Lees, 2000). Viral contamination of shellfish has been proposed as a useful sentinel indicator of human sewage contamination in coastal waters (Asahina *et al.*, 2009; Nenonen *et al.*, 2008).

The risk of viral contamination may be compounded by shallow waters and poor flushing of estuaries in certain areas. Faecal contamination is believed to enter via influxes of fresh water into the marine environment near the surface, so the depth at which shellfish are grown can be important. For example, mussels which are generally grown in deeper water may be less likely to be contaminated than other species.

Enteric viruses adhere and bind to the particulate matter in the water. Following 4-5 hours bioaccumulation in virus-contaminated water, the virus levels in shellfish can reach >1000 particles per animal (Greening *et al.*, 2003b; Greening *et al.*, 2001; Kingsley and Richards, 2003; Seamer, 2007). Over 90% of enteric viruses can be expelled from shellfish in the faeces and pseudofaeces within 48 hr but some are retained and may become sequestered in the shellfish tissues (Schwab *et al.*, 2000; Seamer, 2007) protecting them from elimination.

Several studies have examined the localisation of norovirus in oyster tissues. Noroviruses were detected in the gills, stomach, digestive diverticula and cilia of the mantle (Wang *et al.*,

2008a; Wang *et al.*, 2008b). Recent research has shown that noroviruses can bind specifically to antigens in the oyster gut which are similar to human blood group antigens (HBGA) (Le Guyader *et al.*, 2006a; Tian *et al.*, 2006), and can be internalised within cells of both digestive and non-digestive tissues (McLeod *et al.*, 2009) which could explain why viruses persist after depuration.

The properties and stability of bacteria and viruses are very different; enteric viruses are tolerant of environmental stressors and are known to survive in the environment for several weeks or even months whereas bacteria are depurated from shellfish and die within a few days. For these reasons bacterial indicators of shellfish quality are inadequate for assessment of viral contamination (Lees 2000). An alternative that has been proposed is to use the presence of F-RNA bacteriophage (a coliphage) to indicate the presence of *E. coli* and hence potential faecal contamination (from humans and/or animals). This option was studied in New Zealand but correlation with norovirus presence was not found ((Ball *et al.*, 2008) and see Section 3.1.1).

The heat treatments commonly used to cook shellfish do not inactivate noroviruses. Steaming or freezing for storage is also unlikely to inactivate enteric viruses. An outbreak investigation in Florida in 1995 found that oyster eaters who reported eating only thoroughly cooked oysters (grilled, stewed or fried) were as likely to become ill as those who ate raw oysters (McDonnell *et al.,* 1997). Norovirus has been shown to retain infectivity after incubation at 60°C for 30 min (Dolin *et al.,* 1972).

In the UK during the 1970s and 1980s, a number of outbreaks of gastroenteritis and hepatitis were linked to commercially cooked cockles. Investigation suggested that the batch cooking procedures in use were undercooking shellfish when environmental temperatures were low and shellfish were insufficiently warmed prior to cooking. Research following these outbreaks, using hepatitis A virus, lead to a subsequent recommendation by the UK Ministry of Agriculture for commercial cooking operations was that internal shellfish meat temperatures should be raised to 90°C and held at that temperature for 1.5 min. However, such heat cook parameters may be difficult to reliably achieve for shellfish cooked in large batches without rendering some shellfish unpalatable. Consequently, continuous flow machinery was designed for high throughput operations capable of reliably delivering the above heat cook parameters to all shellfish. Since human noroviruses cannot be cultivated, heat inactivation data for these viruses were not available. However, studies carried out on feline calicivirus, a possible model for noroviruses, suggested that norovirus was more readily inactivated than hepatitis A virus. It was therefore considered probable that approved commercial heat treatment processes based on the heat cook parameters of raising the internal temperature of shellfish meats to 90°C for 1.5 min would effectively inactivate noroviruses (European Commission, 2002).

The NZFSA allows post harvest treatment of norovirus contaminated oysters where there is a validated norovirucidal step. Options for such treatments are given in EC Regulation 853/2004 Annex 1 Section VII, Chapter II.A.5 (http://www.food.gov.uk/multimedia/pdfs/h2ojregulation.pdf). One option is the 90°C for 90 seconds as above.

However, studies in New Zealand of the heat inactivation of noroviruses in the larger sized green mussels (compared to smaller cockles) have indicated that under steaming or boiling conditions inactivation of norovirus is unlikely to be achieved within reasonable domestic

cooking times. After 300 seconds steaming the internal temperature of mussels had not reached 90°C, and longer cooking times were thought unlikely in view of the deterioration of the food. In boiling water an internal temperature of 90°C was reached after 170 seconds, and thus a total time of 260 seconds would be required to inactivate norovirus. However, all mussel shells had opened after 210 seconds, and it was considered that once the shells had opened the mussels would be consumed (Hewitt and Greening, 2006).

A reduction in norovirus titre was not observed in marinated mussels after 4 weeks (Hewitt and Greening, 2004).

2.4   Exposure assessment

2.4.1   The Occurrence of Norovirus in New Zealand Mollusca (Raw)

Information on the virological quality of feral shellfish has been obtained in New Zealand through surveys and monitoring programmes.

A pilot study was conducted in 1999 into the prevalence of viral pathogens in feral and farmed New Zealand shellfish (Scales *et al.,* 2000). From 17 samples, this survey found norovirus in one commercial oyster sample, and in one sample of oysters from recreational gathering, a total prevalence of 12%. This small sample size needs to be interpreted cautiously, especially as the methods for virus detection were less sensitive and robust at that time. The samples were principally from Northland.

Multi-site study (Greening and Lewis, 2007)

Comprehensive data on the prevalence of enteric viruses and bacteriophage in New Zealand shellfish was collected in a two year research study carried out from January 2004 to February 2006. The study aim was to examine the relationship between the occurrence of F-RNA phage (a potential indicator of faecal and viral contamination) and enteric viruses in shellfish, and to determine whether local shellfish were contaminated with human enteric viruses from sewage

Oysters, pipi, cockles, and mussels were collected monthly or bimonthly from 28 sites around New Zealand, including harvesting sites and several sites downstream from a sewage outfall. The sites were located in Dunedin, Napier, Kaipara, Kerikeri, the Bay of Islands and Whangaroa. Over the study period, 360 shellfish samples were collected and analysed. Of these, 174 (48.3%) were positive for one or more human enteric viruses. Shellfish from all but two sites were contaminated with human viruses on occasions. F-RNA phage was detected in 211/318 (66.3%) shellfish samples, but their presence was not clearly associated with the presence of viruses. No correlation between the occurrence of phage and viruses was observed except in an area where shellfish were growing in close proximity to a sewage outfall. The study showed that shellfish beds were occasionally contaminated with human viruses over the two year period, and that shellfish could be unsafe to eat at these times**.**

Microbiological quality of shellfish in estuarine areas (Scholes et al., 2009)

A multi-agency project to investigate the microbiological and virological quality of shellfish in Tauranga Harbour was carried out. Over one year from October 2007- September 2008, 72 non-commercial bivalve shellfish samples were collected monthly from six sites around the

harbour. Shellfish types included oysters, horse mussels, cockles, and pipis. Two pollution events were also monitored during the year - a point source sewage discharge event in February 2008 and then a rainfall event in April/ May 2008. Noroviruses were detected in 23/72 (32%) samples during monthly surveillance, with urban sites more likely to be contaminated with noroviruses. Noroviruses were detected in 19/25 (76.0%) shellfish samples following the sewage discharge, and were present in shellfish growing within 50m of the discharge site for up to 3 months. The overall prevalence of norovirus in shellfish was 49/137 (35.8%), including samples collected after the 2 adverse events.

Local authority monitoring

In February 2008, Northland Regional Council instituted a virus monitoring programme in the Bay of Islands area. Recreational/ non-commercial shellfish are collected monthly from 4 sites previously included in the multi-site study described in Section 5.1.1, and tested for presence of noroviruses and adenoviruses. To June 2009, noroviruses have been detected in 10/44 (22.7%) samples analysed. In 2008 in the same area, 17 shellfish samples were collected in May, October and November by the District Health Board and analysed for noroviruses to determine the status of the Waikare Inlet for re-classification as a commercial growing area. Norovirus was not detected in these samples and has not been detected in shellfish from this area since August 2008. *Ad hoc* viral analysis of oysters has also been carried out in the Whangaroa and Kaipara areas in the last two years and norovirus was detected in 1/8 (12.5%) samples from these sites.

In Christchurch a new ocean outfall for the Sewage Treatment Plant is due to be commissioned within the next few months. In May 2007, Christchurch City Council commenced sampling of shellfish collected from estuarine and coastal sites to obtain 'pre-ocean outfall' baseline data for microbial and viral contamination. Heavy viral contamination was recorded such that the District Health Board erected signs to warn the public of the hazard. Of 74 tuatua and cockle samples, 66 (89.2%) were contaminated with norovirus, occasionally to levels of >1000 and >10,000 norovirus RT-PCR units per gram of shellfish gut.

2.4.2   Quantification of noroviruses in New Zealand shellfish

Estimates of norovirus levels in New Zealand shellfish samples obtained from real time RT-PCR data are shown in Figure 2. The shellfish analysed for norovirus presence were from both non-commercial and commercial sites around New Zealand, but were not related to outbreaks. Norovirus levels are generally low but in recreational shellfish from a few areas they have been extremely high ((Greening and Hewitt, 2008); Data from ESR Environmental and Food Virology Laboratory, unpublished).

Figure 2: Norovirus levels in New Zealand shellfish samples*
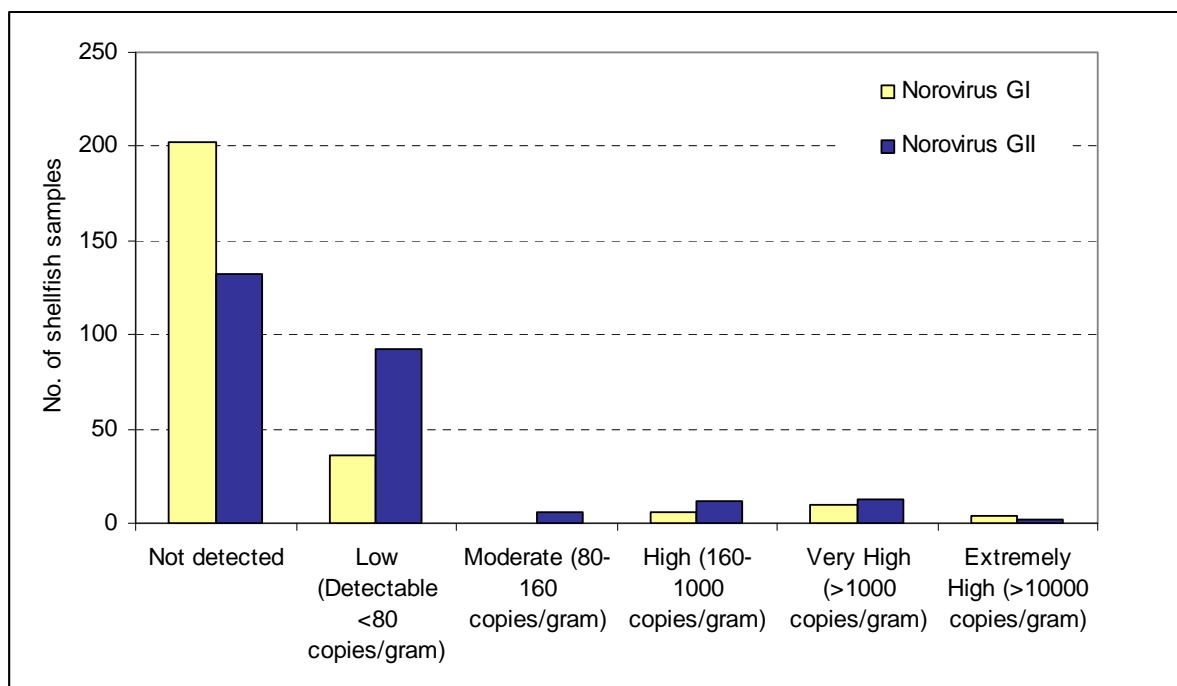
* Analysed by ESR IANZ accredited method for norovirus detection in shellfish (N =257).

2.4.3   Prevalence data from overseas

Overseas data on the prevalence on norovirus in shellfish are summarised in Appendix 1. The prevalence is highly variable (0-76%), which is likely to reflect the fact that local conditions, particularly faecal contamination sources of growing waters, are important. As such, the overseas data are not informative regarding the prevalence that might be expected in New Zealand.

2.4.4   Food consumption: Mollusca (raw)

Proportion of population consuming shellfish

The following information is taken from the New Zealand National Nutrition Survey (NNS) conducted in 1997 (Russell *et al.,* 1999) and the 2002 Children's National Nutrition Survey (CNS) (Ministry of Health, 2003).

For the adult New Zealand population, 2.4% reported consuming shellfish in the previous 24-hour period. Using data from the qualitative food frequency questionnaire (QFFQ), administered as part of the NNS, a higher estimate of 4.0% is obtained. However, 38% of respondents reported 'never' eating shellfish, while a further 39% reporting consuming shellfish less often than once per month.

Those aged over 65 years of age are less likely (1.7%) to consume shellfish than those aged under 65 years of age (2.6%). Children aged 5-15 years are infrequent consumers of shellfish, with only 0.5% of respondents in the 2002 CNS reporting consumption of shellfish in the pervious 24-hour period. The qualitative food frequency questionnaire (QFFQ), administered as part of the CNS suggests a much higher frequency of shellfish consumption of approximately 7%. However, it appears that the definition of shellfish may include crustacean, as well as mollusca. Almost 60% of respondents in the CNS reported never eating shellfish.

A FSANZ assessment of the 1997 NNS data, using a series of standard recipes to determine quantities of commodities in compound food, estimated the proportion of respondents consuming mussels, oysters and scallops as 1.9, 0.6, and 0.3% respectively. The FSANZ assessment of the 1997 NNS data reported a median amount eaten by consumers of 38.4, 45.9, 54.4 g/day respectively for mussels, oysters and scallops.

There is evidence to suggest that certain ethnic groups in New Zealand (Maori, Pacific Islanders, Asians) comprise a greater proportion of the population involved in non-commercial harvesting of shellfish (Hay *et al.,* 2000). Kaimoana, harvested by Maori, particularly in the upper North Island, is an important cultural and dietary component. A survey in this region found that 11% of households reported collecting seafood more than once a week, 31% collected seafood at least weekly, and 52% reported collecting seafood at least fortnightly (Hay *et al.,* 2000).

Mean daily consumption of shellfish

Analysis of all (raw and cooked) shellfish serving data from the 1997 NNS gave a mean daily intake for consumers of shellfish of 106 g/person/day and a mean across the whole study population (consumers and non-consumers) of 2.5 g/person/day. The corresponding data for the child population (5-15 years) gave a mean daily consumption for consumers only of 49 g/person/day and for all respondents of only 0.2 g/person/day.

Analysis of the data from the 1997 NNS suggests that Maori consumers, on average, consume larger amounts of shellfish (average daily consumption of 160 g as compared to 113 g for non-Maori), although the data available do not suggest that they eat shellfish more frequently. These data represent a national average; as described in the preceding section, frequency of consumption in some regions is much higher.

These figures are comparable to those obtained in the Life in New Zealand (LINZ) survey (the previous National Nutrition Survey) which reported mean intakes of shellfish of 70 g/day for all males, 99 g/day for all females, and 84 g/day for all consumers. The percentages of respondents consuming shellfish were slightly higher in the LINZ survey (4% of males and 3% of females).

Serving sizes for shellfish consumption

Analysis of data from the 1997 NNS gave mean, median and 95th percentile serving sizes for shellfish of 92.3, 64.0 and 276 g. Child servings, as reported in the 2002 CNS are smaller, with corresponding values of 49.4, 43.5 and 108 g. These values are derived from all shellfish servings, whether raw or cooked. There are insufficient data to differentiate raw versus cooked servings, and serving size is probably independent of cooking status.

Types of shellfish consumed and cooking method used

Of 128 servings of shellfish identified in the 1997 NNS 24-hour dietary recall records, 59 (46%) were mussels, 22 (17%) were oysters, 15 (12%) were scallops and 13 (10%) were paua. The balance was pipis, tuatua or recipes in which the shellfish was not specifically identified. Oysters were the shellfish most commonly consumed raw (13/22 – 59% of servings). Mussels were consumed raw (7/59) or marinated (21/59) for 47% of servings.

There is a data gap concerning exposure assessment from shellfish, in that while recreational gathering of feral shellfish is acknowledged to be widespread, there are few quantitative consumption data.

2.4.5   Evaluation of exposure

There are two important factors affecting the exposure assessment for noroviruses in shellfish. One is that the viruses concentrate in the gut which is a small part of the whole animal, and they are not distributed throughout the flesh. Often the gut is excised for testing purposes, and gut weight may not relate to the flesh weight. Thus viral concentration is unrelated to shellfish size, or serving size. Secondly, usually multiple shellfish will be consumed in a meal. Individual shellfish may or may not contain viruses, and the number of viruses in individual contaminated shellfish will vary. Thus an exposure assessment based on amount eaten and putative uniform concentration of the hazard is not suitable. Instead, a stochastic process based on the number of shellfish eaten, probability of contamination, and a distribution of concentrations would be required.

Number of servings

From a national perspective, consumption of shellfish in New Zealand is relatively uncommon with only 2.4% of the population reporting consumption of shellfish on any particular day. Based on limited data and anecdotal comments, consumption of recreationally gathered shellfish for personal use is likely to be concentrated in coastal regions, particularly in the north of the North Island.

For calculating the number of shellfish servings consumed per annum in New Zealand the population was divided into three subgroups:

Less than 5 years - no information is available on shellfish consumption by this group and it was assumed that they are non-consumers of shellfish
5-15 years - the numbers of servings for this group were taken from the CNS02
15+ - the number of servings for this group were taken from the NNS97

The number of people in each age group was calculated using current national estimates of total population (4,320,000) and the proportion of the population in each of these age ranges at the 2006 Census. This approach will underestimate older age groups and overestimate the younger age groups, as the age profile of New Zealanders is moving with time to an increasingly aged population.

Less than 5 years.
Estimated population: 289,000

Total servings: 0

5-15 years
Estimated population: 688,600
Survey (CNS) population: 3275
Total survey shellfish servings: 16
Estimated annual servings: 1.23×10^6

15+ years:
Estimated population: 3,342,400
Survey (NNS) population: 4636
Total survey shellfish servings: 128
Estimated annual servings: 3.37×10^7

Total annual shellfish servings: 3.49×10^7

According to data from the Nutrition surveys, 18% of these servings would be consumed raw (approximately 6 million servings).

Frequency of contamination

Recent surveys and monitoring programmes carried out in New Zealand have shown that prevalence of contamination of feral shellfish can be high (up to 90%) (Section 2.4.1), but fluctuates both regionally and temporally (e.g. 25% of non-commercial shellfish samples from the Bay of Islands (Waitangi Estuary, Te Haumi Point, Waikare Inlet Lease 64 and Kawakawa river (top) site) were contaminated but none of the commercial shellfish samples from the same sites were contaminated). There are fewer data on commercial shellfish, but there is considerable information linking outbreaks of illness with consumption of commercial oysters both grown locally and imported (see Section 6.1.2).

Predicted contamination level

Norovirus levels in commercial and non-commercial shellfish are generally low (<80 copies/ gram) (see Figure 2). Feral tuatuas from the Christchurch region have been found to contain high levels of norovirus (Greening, unpublished data).

The dose-response relationship for norovirus (Section 4.4) means that very low levels of contamination in a food will provide a high probability of infection.

Growth rate during storage and most likely storage time

Noroviruses are unable to replicate outside the human body and, consequently will not grow in shellfish during storage.

Heat treatment

Of the servings of shellfish identified in the 1997 National Nutrition Survey, 57% identified the product as being cooked, 20% raw, 19% marinated, 3% canned and 2% smoked. Mussels and oysters were the most commonly consumed shellfish types, and approximately half of the servings of these shellfish were reported as raw or marinated.

However, as this risk profile is for raw shellfish, thermal inactivation through a cooking process is not relevant. A proportion of cooking methods for shellfish will use light heating (e.g. steaming) which is unlikely to inactivate norovirus.

Exposure summary

Raw molluscan shellfish are an infrequently consumed food in New Zealand, when considered as part of the national consumption overview. However, consumption of recreationally gathered shellfish is likely to be concentrated in certain regional and ethnic populations, and so exposure will occur mostly in those populations.

There is accumulating data to indicate frequent contamination of New Zealand shellfish (both commercial and feral) with norovirus. Even low levels of contamination will present a high probability of infection.

3   EVALUATION OF ADVERSE HEALTH EFFECTS

3.1   Disease Characteristics

Outbreaks of noroviral gastroenteritis occur world-wide. Symptoms develop within an average incubation period of 12-48 hours (CDC, 2001).

The main symptoms are vomiting, often projectile, which is present in >50% of cases, diarrhoea, stomach cramps, abdominal pain, low-grade fever and headache. The illness is generally mild and self-limiting, with a duration usually between 24-72 hours.

Following infection, noroviruses are shed in high numbers ($> 10^{8}\text{-}10^{9}$/g) in stools (Atmar *et al.,* 2008). Noroviruses may also be shed in vomit, and transmission of disease through aerosol droplets has been reported (Marks *et al.*, 2000).

Attack rates are high, generally >30-50%, and sometimes as high as 80%. Immunity is not sufficiently cross-reactive to protect against different norovirus strains (D'Souza *et al.,* 2007). Immunity appears to be short lived (i.e. 6-14 weeks) and subjects who were symptomatic could be re-infected when challenged 2-3 years later with the same inoculum (Patel *et al.*, 2009). Norovirus infection affects all age groups, but the elderly and the immunocompromised are particularly susceptible.

Fatalities have been reported following norovirus infection; an analysis of reported mortality amongst the elderly (>65 years) in England and Wales suggested that 20% of deaths caused by infectious intestinal disease (other than *Clostridium difficile*), and 13% of deaths casued by non-infectious intestinal disease were associated with norovirus infection (Harris *et al.*, 2008). These may result from severe dehydration. No long term sequelae have been reported (CDC, 2001). Treatment is not usually provided but patients may need rehydration.

There is evidence that susceptibility to norovirus infection is linked to genetic factors, particularly the human blood group antigens (HGBA), which include the H, Lewis, and A histo-blood group antigens (Hutson *et al.*, 2004). These structurally related carbohydrates occur on glycolipids and glycoproteins that are found on the exterior cell surface. Norovirus bind to HGBA as part of their attachment to cells. The strongest level of binding appears to be with H type 1 (also known as Lewis (d)) HGBA. Individuals may express (secrete) Lewis antigens which can be detected in saliva, and Lewis positive individuals were those who were infected in the challenge trail to develop a norovirus dose-response relationship (Teunis *et al.,* 2008). The carbohydrate binding of GI noroviruses appears to be linked with Lewis (d) secretor status, and 80% of Northern Europeans and Caucasian Americans are secretor positive (Hutson *et al.*, 2002). However there does not appear to be a similar relationship for GII strains and host HBGA (Halperin *et al.*, 2008). The correlation between norovirus genogroup binding and the many HGBA types, as well as the HGBA status of populations, need further investigation before it can be used for risk assessment.

3.2   Virus Shedding

Virus shedding by infected humans is important for the potential contamination of food and fomites. The duration of the virus shedding period has been observed to be up to several weeks (Atmar *et al.,* 2008). In this volunteer study, 11 of 16 secretor-positive (i.e. susceptible) persons inoculated with norovirus met the case definition for viral

gastroenteritis. Symptomatic illness lasted 1-2 days. Peak virus titre levels occurred at 2-5 days after inoculation. Virus shedding was first detected using RT-PCR 18 hours after inoculation, and lasted 28 days (range 13 – 56 days).

Several studies have reported excretion of noroviruses for long periods following infection (Murata *et al.*, 2007; Siebenga *et al.*, 2008). A study of children found that virus was shed in faeces for up to 100 days after resolution of disease symptoms (Kirkwood and Streitberg, 2008). However, the significance of prolonged virus excretion in disease transmission cannot be determined until methods to assess norovirus infectivity are available.

3.3   Dose Response

In 2008, a dose-response relationship for a norovirus strain (Norwalk virus, GI.1) derived from a human challenge trial was published (Teunis *et al.,* 2008). The dose response relationship was based solely on the response of susceptible secretor positive volunteers (see Section 3.1). Both infection (excretion of the virus in faeces) and illness (appearance of symptoms) were investigated.

The dose-response relationship for infection shows that ingestion of very low numbers of virus particles causes a high probability of infection (approaching $p = 0.5$ for a single virus particle). However, the dose response relationship did not reach $p = 1$ even at the highest dose, suggesting that a fraction of the exposed secretor-positive population had low susceptibility, attributed possibly to acquired immunity.

The conditional probability of illness among infected subjects showed dose dependence, and was also steep at low doses (overall, out of 22 infected subjects 15 (68%) developed acute gastroenteritis symptoms). The authors comment that these results suggest that a high risk event, where heavy contamination occurs, may produce not only many cases of infection but also many cases of illness. Conversely, if a person is infected by a low dose, the probability of illness was also low. Thus low levels of endemic exposure (e.g. contaminated drinking water) may lead to infection but relatively low numbers of illnesses, thereby lowering the potential for recognition of contamination incidents.

A recent analysis suggests that attack rates for oyster associated outbreaks are higher than those for food-handler associated outbreaks (Noda *et al.,* 2008). This difference may be due to the accumulation of multiple genotypes by oysters, whereas contamination by a food handler is likely to involve only a single genotype. This was supported by analysis of genotypes found in outbreak cases from each source. There are many observations of multiple norovirus strains in shellfish related outbreaks, including New Zealand outbreaks (Simmons *et al.*, 2007; Simmons *et al.*, 2001).

3.4   New Zealand Outbreak Information and Human Health Surveillance

Gastroenteritis specifically caused by norovirus infection is not a notifiable disease and public health service providers currently only report outbreak data to the surveillance system. Elevating norovirus infection to notifiable disease status is proposed under revisions to the Public Health and Disability Act 2000, which are pending.

In a 2005 survey of New Zealand community and hospital laboratories (King *et al.,* 2007) 2/35 reported testing for norovirus (excluding the Norovirus Reference Laboratory). Sample

numbers tested for viruses were a very small proportion of the total (approximately 10% of samples were tested for rotavirus, about 1.5% for adenovirus, and less than 0.5% for norovirus). The same survey estimated that for approximately 80% of faecal samples provided by patients with symptoms, a pathogen is not detected.

3.4.1   Norovirus data from reported outbreaks

The number of reported outbreaks and cases of norovirus infection from 2001-2007 are given in Table 3. These data are taken from the ESR Annual Outbreak Summaries, from surveillance data recorded in the database EpiSurv (http://www.surv.esr.cri.nz/surveillance/annual_outbreak.php). Norovirus is the most frequently reported agent for outbreaks in New Zealand, in terms of both numbers of outbreaks and numbers of cases.

Table 3: Number of reported outbreaks (O/B) and cases of norovirus infection 2001-2007

2001		2002		2003		2004		2005		2006		2007	
O/B	cases	O/B	cases	O/B	cases	O/B	Cases	O/B	cases	O/B	cases	O/B	cases
45	541	73	1263	73	1368	126	3022	61	1159	156	3945	206	5902

NB: in 2001, Norovirus was categorised as 'Norwalk-like-virus (norovirus); O/B – outbreak.

Individual norovirus outbreak records from EpiSurv from 2001-2007 have also been reviewed. Due to data cleaning (particularly review of the assignment of transmission routes based on information in comments fields), this summary does not always correspond exactly with that given in the annual outbreak summaries. There were 809 reported outbreaks of norovirus infection including a total of 18,508 cases. Of these, 2,623 cases were recorded as confirmed; 1,779 cases were laboratory confirmed (norovirus detected in a faecal sample) and 13,805 were probable cases. The lower number of laboratory-confirmed cases is due to the fact that faecal specimens are not collected from all cases and only a proportion of specimens are analysed for norovirus presence. Norovirus confirmation is recorded by outbreak rather than by case and is often not retrospectively updated in EpiSurv.

Mortality recorded for reported outbreak cases of norovirus infection has increased in recent years. From 1997 to 2005, a total of 6 deaths were reported, whereas in 2006 and 2007 the numbers were 5 and 10 respectively. In both 2006 and 2007 it was noted that all the fatalities related to outbreaks in residents of rest homes or hospitals with continuing care, which suggests that there were other contributing factors as well as the norovirus infection.

The increase in numbers of reported norovirus outbreaks and mortality in recent years will at least partly be due to improved detection capability.

From the total number of outbreaks, 19.9% (161/809) were associated with environmental sources; 17.6% (142/809) were associated with foodborne infection and 61.0% (494/809) were associated with person-to-person transmission. Several of these outbreaks were associated with both person-to-person transmission and foodborne sources.

Examining the EpiSurv reported outbreak data more closely, although 22 of the 142 foodborne outbreaks were described as laboratory linked with both the patient and the source

(i.e. norovirus detected in both a faecal sample from at least one case and a source), a source vehicle was listed for only 16 of these. Eleven of the 16 were linked to consumption of oysters.

The Norovirus Reference Laboratory, in addition to analysing faecal specimens, consolidates information on outbreaks, which can provide a more detailed picture of the epidemiology. This enables a larger number of outbreaks to be identified, and outbreaks initially reported as gastroenteritis can be assigned as norovirus.

Data collected by the Norovirus Reference Laboratory show that between 2001 and 2007, shellfish (mainly oysters) were implicated in 29 outbreaks of gastroenteritis caused by norovirus (GE Greening, unpublished data). Of these, imported oysters were associated with 11 outbreaks and New Zealand oysters implicated in seven outbreaks. For the remainder, information on oyster source was not available. This number of shellfish linked outbreaks is higher than that estimated only from the data reported to EpiSurv above.

A number of investigations into specific norovirus outbreaks linked to shellfish have been reported, and these are summarised below.

Between November 1994 and January 1995, three gastroenteritis outbreak investigations in Northland were linked to consumption of Pacific oysters harvested in November 1994 from the Waikare Inlet of the Bay of Islands (Jarman and Brown, 1995). A viral aetiology was suspected and was supported by norovirus detection in the stools of one patient. Northland Health recalled the shellfish products.

In December 1994, 36 (38%) of 95 people attending a yacht club party developed gastroenteritis. Epidemiological and microbiological investigations indicated that oysters contaminated with norovirus were the most likely cause of infection. The oysters were believed to be harvested from a Bay of Islands oyster farm (Jones and Graham, 1995).

A detailed study of 18 outbreaks of norovirus infection was carried out by Wong *et al.* (Wong *et al.,* 1997). One outbreak was attributed to imported Chilean oysters based on a retrospective cohort epidemiological study. In total, seven outbreaks of acute gastroenteritis between June and December 1996 were associated with oysters imported from Chile (Bates, 1997). Investigations were carried out for four of these outbreaks, there was insufficient evidence to draw conclusions for two outbreaks, and there was strong evidence implicating imported Chilean oysters for the other two outbreaks. Human enteroviruses were detected in the oysters, indicating faecal contamination. No methods for norovirus detection in shellfish were available at the time.

A retrospective analysis of New Zealand norovirus outbreaks occurring between 1997 - 1999 found that food was the predominant mode of transmission in 27/50 (54%) outbreaks. A specific food or food type was epidemiologically implicated in 12 of these 27 outbreaks. The food type most commonly associated was seafood (5/12 (42%) outbreaks), and shellfish were implicated in three of these (Greening *et al.,* 1999).

A series of ten outbreaks of norovirus gastroenteritis in Auckland in 1999 occurred amongst people who had consumed raw Pacific oysters (Simmons *et al.,* 2001). Of 326 people attending common events associated with the outbreaks, 86 cases were identified and 32 were laboratory confirmed. Three outbreaks were not able to be analysed due to insufficient number of cases, and in two outbreaks oyster consumers were not significantly more likely to

develop illness than non-consumers. In the remaining five outbreaks, oyster consumption was a statistically significant risk factor.

Traceback identified the source of the oysters as from the Northland region. Norovirus strains indistinguishable from those identified in some cases were identified in remaining oysters from some batches. No potential contamination events (e.g. heavy rainfall) were identified in the growing areas. Noroviruses were identified in two batches of oysters harvested from different growing areas and implicated in four of the outbreaks. Sewage effluent from recreational boats was identified as the likely source of contamination in growing waters at one site. Contamination by infected workers through the processing and supply chain was thought unlikely, as no history of illness was identified amongst people involved. This was the first New Zealand report to identify norovirus contamination in commercially harvested shellfish and to link their occurrence with outbreaks of viral gastroenteritis.

In August 2001 three outbreaks of acute gastroenteritis involving 24 people were notified to Auckland District Health Board (Jones and Simmons, 2001). Norovirus was identified as the likely pathogen and fresh, raw Pacific oysters were determined to be the probable source of illness. Six of the 12 (50%) faecal samples obtained from cases in these outbreaks tested positive for norovirus; no other pathogens were identified. The oysters were identified by traceback through the harvesting, processing, and distribution chain to two growing areas in Northland (the Waikare Inlet and Orongo Bay). Norovirus was not detected in any of the three batches of oysters associated with these outbreaks. However, oysters harvested from the Waikare Inlet a week earlier and associated with a Northland case tested positive for norovirus. A source of contamination was not identified through the processing and distribution chain. However, the two growing areas were closed for a period of 21 days. This was the third cluster of norovirus outbreaks implicating oysters harvested from the Waikare growing area since 1994.

As a result of these virus contamination events, the regulatory authorities made changes to the growing area classification for the Waikare Inlet growing areas. Parts of the area were reclassified as "Restricted" – whereby oysters must be processed by an approved method before consumption. NZFSA conducted an international review of relaying norovirus contaminated BMS and was unable to find a country that specifically allowed this post harvest treatment. However, after considering the international advice received NZFSA approved relaying, providing the relay period was for 60 days and the oysters were a minimum of 300m distance from other oysters (the distance has since been amended). Upgrades to septic tanks, the sewage treatment plant, identification and repair of leaking sewage pipes, and improved boating controls have led to improvements to the water quality in the area over the last five years. The Waikare Inlet has now been reclassified from "Restricted" back to its original classification of "Conditionally Approved".

Following these viral contamination events, Hong Kong authorities temporarily refused entry of farmed North Island Pacific oysters for several months from March 2001. A sampling programme for New Zealand oysters on arrival before clearance was established. Several batches were tested and virus was not detected. Hong Kong currently allows the importation of all New Zealand product except from the Waikare Inlet. US authorities have also reported norovirus illness in Hawaii from New Zealand shellfish, resulting in recalls of product. These events caused the closure of major oyster growing areas in Northland and the loss of several million dollars worth of exports.

In July 2004, four outbreaks of gastroenteritis were associated with consumption of fresh raw oysters harvested from the Auckland area. Norovirus was identified in faecal specimens and also in two samples of oysters from the lease. A site inspection and review of classification of the oyster growing area found no clear source for the norovirus contamination; however some potential risk factors were identified including septic tank discharges, discharges from the nearby river and discharges from passing boats. Genotyping of the norovirus strains obtained from one oyster sample and from faecal specimens showed them to be indistinguishable, which established the association between the oysters and the various outbreak-linked events.

In 2006 an outbreak of norovirus infection amongst people attending an international rugby test match at Eden Park was linked to imported South Korean Pacific oysters (Simmons *et al.,* 2007). Three previous and four subsequent outbreaks of gastroenteritis in other parts of New Zealand during 2006 were also linked with raw or lightly cooked Korean oysters. Labelling on the oysters advising cooking prior to consumption was ignored by the caterers. As a result of these incidents imports of Korean oysters were suspended by the NZFSA.

In July 2008, Auckland Regional Public Health Service was notified of several gastroenteritis incidents affecting 121 people and a norovirus outbreak was suspected. An outbreak investigation, consisting of an epidemiological investigation, oyster traceback, virological analysis and several environmental surveys showed a link with consumption of raw New Zealand Pacific oysters grown and marketed locally in the Auckland area (Grey *et al.,* 2009). The implicated lease was the same one associated with the 2004 outbreaks. Seven separate outbreaks linked to consumption of these oysters were recorded on EpiSurv. The two largest outbreaks occurred following functions where raw oysters had been served in a buffet meal. Raw oysters were the only foods significantly associated with illness. Traceback implicated oysters grown on one lease or relayed through it from other leases.

Norovirus was identified in faecal specimens from cases and oysters. Fifteen faecal samples (94%) were positive for a novel recombinant strain of norovirus GII (GII.c-GII.12) and 14 oyster samples (61%), were positive for norovirus genogroup II. A norovirus strain from one oyster sample was indistinguishable from the recombinant GII.c-GII.12 strain identified in the faecal samples. The implicated growing area was closed and a product recall of all oysters harvested from the leases was initiated. Fifteen further notifications related to consumption of these oysters were subsequently reported.

The source of the contamination has not been identified but a number of potential sewage contamination points were identified along a river upstream from the main lease implicated in the outbreaks. Another potential source of contamination was identified at the oyster farm factory where the disposal field for the factory's sewerage treatment plant had become saturated following heavy rainfall, resulting in wastewater seeping from the field into a nearby creek. The creek water was used to 'wash down' freshly harvested oysters on receipt from the implicated growing area prior to shucking. Prior to re-opening, the growing area was required to comply with the three pronged process from the Regulated Control Scheme-Bivalve Molluscan Shellfish discussed under Regulatory Food Controls.

The 2004 and 2008 outbreaks associated with New Zealand oyster consumption both occurred in July in product harvested from the same lease. Oysters and water met microbiological safety criteria, although heavy rainfall events had occurred previously in the vicinity of the growing area.

Another small outbreak involving oysters grown in Kerikeri was reported in 2008. Norovirus was identified in samples from both cases, as well as oysters from the growing area. The growing area was closed according to NZFSA regulations.

3.5   Adverse Health Effects Overseas

Overseas outbreaks of norovirus infection have frequently been associated with shellfish consumption, as shown by the information summarized in Appendix 3. These outbreaks confirm the importance of oysters as the most commonly implicated type of shellfish, and that almost all outbreaks are from oysters consumed raw.

3.6   Adverse Health Effects Summary

Since infection with norovirus is not a notifiable disease in New Zealand the available data derives from reported outbreaks (elevating norovirus infection to notifiable disease status is proposed under revisions to the Public Health and Disability Act 2000). Most outbreaks are not investigated in detail, and for those that are investigated a vehicle is often not identified. Given these limitations, the number of outbreaks in Section 3.4.1 where both imported and New Zealand produced oysters are confirmed as the vehicle represents strong evidence for the risk of norovirus infection from consumption of this type of shellfish.

4 EVALUATION OF RISK

4.1 Existing risk assessments

A preliminary quantitative risk model for norovirus in shellfish was developed as part of the multi-site study (Greening and Lewis, 2007) described in Section 2.4.1. This used information on viral contamination, shellfish consumption, and cooking methods and their effects on viruses. The additional quantitative norovirus data now available for both commercial and non-commercial shellfish in New Zealand, and the recent publication of a dose-response relationship for norovirus (Teunis *et al.,* 2008) would assist further development of the model.

Scientists from the Netherlands have written a Risk Profile of norovirus in bivalve molluscan shellfish for the Codex Committee on Food Hygiene (CX/FH06/38/10, available from: ftp://ftp.fao.org/Codex/ccfh38/fh38_10e.pdf). This ten page Profile identified the need for internationally standardised testing procedures for noroviruses in shellfish, and that RT-PCR detection of norovirus does not provide evidence that the virus is infectious. Recommended risk management actions were to reassess depuration procedures, and the importance of controlling sewage discharges from boats.

4.2 Economic Costs and Burden of Illness

In New Zealand it has been estimated that there is a mean of 403,000 norovirus infections annually (5 and 95 percentiles 71,000 – 1,004,000) (Cressey and Lake, 2007). This represents 536 disability adjusted life years (DALYs). From an expert elicitation process the most likely proportion foodborne was 39.6% (minimum 27.9%, maximum 48.9%). This then provides an estimate of 210 DALYs for foodborne infections. This burden of illness was second (after foodborne campylobacteriosis) in a risk ranking of potentially foodborne diseases.

The burden of disease to the health system and society in general has also been considered, through a cost of illness estimate, based on the same incidence data (Cressey and Lake, 2008). This estimated the total cost for norovirus infections as $7.6 million, with foodborne infections costing $3.0 million. Again this was the second highest burden estimate, but much lower than the highest estimate, for foodborne campylobacteriosis, of $74 million.

The expert elicitation process (http://www.nzfsa.govt.nz/science/risk-ranking/FW0563_RISK_RANKING_report.pdf) also estimated the proportion of foodborne disease that was due to specific foods. For the 39.6% of norovirus infections that were considered foodborne, 40% were considered to be transmitted by shellfish (minimum 29.3%, maximum 49.6%). Note that some of the remaining 60% of foodborne norovirus infections were considered to be transmitted by infected foodhandlers to food and then consumers, as this was considered as "foodborne" by the group of experts. Overall, this estimate would imply that approximately 16% of all norovirus infections were transmitted by shellfish as a vehicle.

In the United States it has been estimated that viruses account for 67.2% of the cases of illness caused by known foodborne pathogens; 34.8% of the hospitalisations, and 7.1% of the deaths (Mead *et al.,* 1999). This included noroviruses, rotavirus, astrovirus, and hepatitis A virus. Noroviruses were the most likely to be foodborne (40% of all norovirus infections),

whereas the other viruses were considered infrequently to be transmitted by food (rotavirus 1%; astrovirus 1%, hepatitis A virus 5%).

For Australia it has been estimated that foodborne viruses cause a median of 470,000 of 1,480,000 (32%) cases of foodborne illness in a typical year *circa* 2000 (Hall *et al.*, 2005). This includes norovirus (estimated 25% foodborne), rotavirus (2% foodborne), and astrovirus/adenovirus (10% foodborne).

4.3   Summary of Attribution

Estimates for New Zealand, the United States, and Australia indicate that 25-40% of norovirus infections are foodborne, with 40% of the foodborne infections estimated as being transmitted by shellfish in New Zealand.

The majority of the remaining foodborne transmitted infections are likely to be due to contamination of food by infected food handlers during preparation. This is especially important for foods such as salads, soft berry fruits and delicatessen goods which are generally consumed without further cooking. The exclusion of foodhandlers for 48-72 hours following gastrointestinal illness has been recommended to prevent such contamination (CDC, 2001). Asymptomatic carriage of norovirus by workers in a catering facility on Japan has been reported, but no cases of illness were linked with this finding (Okabayashi *et al.*, 2008). Foodhandlers are not considered to be a major factor in the contamination of raw shellfish.

Person-to-person transmission is probably the dominant route for non-foodborne infections. The importance of healthcare settings, and probable person-to-person transmission of norovirus infection has been highlighted in analysis of data from the United Kingdom. Based on the norovirus outbreaks reported from 1992 to 2000, two epidemiological patterns have been identified (Lopman *et al.,* 2003). Outbreaks in healthcare facilities (hospitals and residential care) comprised 79% of the total. These showed a winter peak, higher death rates and prolonged duration, but were of smaller size and less likely to be foodborne than outbreaks in other settings. Of the 86 foodborne outbreaks identified, the most common foods were oysters (20), salads and vegetables (17), poultry (9), fish (6), meat (5) and "other" (29). Apart from oysters, the contamination of foods was attributed to contamination from infected foodhandlers, and this represented the majority of foodborne outbreaks. As expected, outbreaks in food outlet settings dominated the foodborne transmission route.

Health care settings (hospitals and rest homes) represent over half the settings identified for 935 outbreaks collated by the Norovirus Reference Laboratory from 2001-2007 (for data see Appendix 3). Catered events are the next most common setting, highlighting the importance of foodhandlers.

4.4   Summary of foodborne human health risk

In terms of volume, commercially grown mussels and oysters are the largest segment of the molluscan food supply in New Zealand. Of the feral populations, the commercial cockle harvest appears to be the most important. The volume of imported molluscs is very small in comparison with the domestic supply, but imported oysters have caused norovirus outbreaks in New Zealand.

Based on the expert elicitation attribution estimates in Section 4.2 there may be 16% (approximately 65,000 cases) of norovirus infections transmitted by shellfish each year. However, this estimate has high uncertainty, as the estimates of both attribution and total number of cases have wide confidence intervals.

The regular identification of outbreaks of infection linked to contaminated oysters over the past 15 years indicates an ongoing risk, and raw oysters are the most commonly identified vehicle. Mussels and scallops have not been identified as causing outbreaks; these types of molluscs are probably of lower risk of contamination due to their occurrence in deeper water (while feral mussels can occur in the intertidal zone, aquaculture mussels are grown in deeper water). Cockles have not been identified as the cause of a norovirus outbreak in New Zealand but shellfish monitoring programmes have found contamination in this type of shellfish.

It is unclear whether the risk of norovirus infection from commercial shellfish for the New Zealand population has changed since the previous Risk Profile was completed in 2003. However, the risk has been better characterised as a result of surveys including the multi-site and Tauranga Harbour surveys, and evidence for widespread norovirus contamination of shellfish, particularly feral shellfish, has been obtained.

Commercial oyster related outbreaks of norovirus infection continue to be identified, particularly in the Auckland region. Although widespread contamination of feral shellfish with norovirus has been demonstrated, surveillance data linking this contamination with human illness have not yet been reported.

Recent improvements in detection methods have facilitated the collection of data to better describe and manage this risk. Reducing sources of human faecal contamination in the Bay of Islands area have demonstrated how the risk can be better managed.

This Risk Profile has identified a number of data gaps which if filled would contribute to the increasing knowledge on noroviruses in foodborne disease, especially BMS in New Zealand. Many of the data gaps cannot be answered until there is a robust method to assess and quantify the infectivity of human norovirus. This is still not possible.

The data gaps are summarised below:

Surveillance
- Improved surveillance to link norovirus cases and outbreaks to a particular food source, in particular BMS consumption.
- The prevalence of the norovirus in key growing/recreational shellfish gathering areas, including the seasonal and geographical distribution of viral contamination.

Exposure assessment
- Gathering of feral shellfish recreationally is acknowledged to be widespread. There is little quantitative data to assess norovirus exposure from both recreational and commercially grown shellfish.
- The role of post-harvest food handlers in the transmission of norovirus in shellfish is unknown.
- Information on the current level of shellfish consumption per person, per meal, per age group, cultural group, etc. in New Zealand.

- Information on the minimum infective dose in shellfish and how it relates to norovirus RNA levels detected by RT-PCR, and also information on dose response.
- Presence and distribution of genetic susceptibility factors for the different norovirus strains in the New Zealand population.
- Information on the survival rates of norovirus in boat and domestic sewage to define the contamination process.
- Information on the survival and persistence of norovirus in the environment and in shellfish. Efficiency of sewage and wastewater treatment processes for removal of norovirus and hepatitis A virus.
- Role and value of microbial and viral source tracking tools for predicting occurrence of viral contamination, especially norovirus contamination.
- Quantitation methods for infectious norovirus in shellfish and the environment. Data based on RNA quantitative estimates of viral RNA present in shellfish does not relate to infectivity. The only measure of infectivity currently available is by human dose response experiments.

Detection Methods
- Improved, efficient norovirus recovery, detection and quantitation methods from shellfish. Current norovirus recovery methods from shellfish are frequently of variable efficiency, which may relate to shellfish type. Accurate estimation of the quantity of virus present in a sample is problematic.

Effectiveness of methods for virus removal from shellfish and control strategies
- Efficiency and effectiveness of virus removal or natural depuration from shellfish in the environment and in post-harvest treatment.
- Information on the effectiveness of depuration and relaying processes preharvest prior to putting shellfish on market.
- Value of testing shellfish for norovirus at intervals following sewage spills and discharges.
- Inactivation mechanisms for norovirus and other pathogenic viruses in shellfish. Data is required on stability and persistence, effect of temperature, pH, time, matrix/organic material, disinfection by chemicals, ultraviolet light and radiation.
- Effectiveness of ultra high pressure processing of shellfish for inactivation of human norovirus. .

Virus recombination
- Significance of norovirus recombination in New Zealand shellfish harvested from contaminated areas. Shellfish often contain a cocktail of viruses and infection with multiple strains may lead to the generation of potentially more virulent recombinant norovirus strains. A novel recombinant strain associated with several outbreaks has already been identified in New Zealand shellfish.

Role of animal viruses
- Information on potential zoonotic transmission of noroviruses between animals and humans through dual contamination events in shellfish. Could animal noroviruses contribute to the generation of new more virulent recombinant norovirus strains infecting humans?

5 AVAILABILITY OF CONTROL MEASURES

5.1 Current control measures

5.1.1 Aquaculture

The Animal Products (Regulated Control Scheme- Bivalve Molluscan Shellfish) Regulations 2006 (SR2006/38), which came into force on 1 June 2006, imposed a regulated control scheme under Part 3 of the Animal Products Act 1999 in respect of the commercial growing, harvesting, sorting, and transporting of bivalve molluscan shellfish. The Animal Products specification for Bivalve Molluscan Shellfish Notice 2006 (BMSRCS Notice) contains the detailed standards for shellfish safety and is notified under the Animal Products Act, it can be found online at: http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal material-product/shellfish/bmsrcsspecv-16_2_signed.pdf

The BMSRCS Notice includes requirements for growing area sanitary survey and classification, relaying, storage, marine biotoxin control, harvesting/sorting/transport, and laboratory testing. The Microbiological Risk Management section (Part 13) specifies the actions to be taken in the event of an outbreak involving two or more persons not from the same household, including potential closure of growing areas for harvesting. If pathogens are detected in the BMS, a risk assessment is performed to determine the appropriate actions. Clause 35(3) is relevant for the relaying of norovirus-contaminated product, Clause 76 relates to illness following consumption and Clause 76(8) specifically refers to actions following contamination events in growing areas and viral pollution. Clause 78 refers to sewage pollution events.

In terms of the assessment of growing waters and shellfish quality, although norovirus is listed amongst the pathogens that may occur, only bacteriological limits are set; for faecal coliform and *E. coli* levels in growing waters and BMS samples respectively.

Pertaining to Clause 76 (8) above, in outbreaks of BMS related illness of viral aetiology (including those attributed to norovirus) the BMSRCS notice prescribes a three pronged approach to the re-opening of the growing area: each source of the pathogen must be identified and fixed; the growing area then remains closed for a further 28 days; and lastly a minimum of 5 samples must be taken from the farm/growing area and show that the pathogen is no longer present. Laboratories conducting analyses required by this notice must comply with provisions in Part 15. Although specific test methods for norovirus detection are not specified, the laboratory is required to have ISO/IEC 17025 accreditation for any pathogen detection method used. This is a unique risk management approach for growing areas implicated in norovirus illness outbreaks.

5.1.2 Post harvest treatments: depuration and relaying

There are two post-harvest processes by which molluscs can be treated to remove norovirus contamination. The Animal Products (Regulated Control Scheme - Bivalve Molluscan Shellfish) Regulations 2006 (SR 2006/38) defines these as follows:

> **relay** means to transfer BMS from a growing area to another growing area for the purpose of reducing pathogens or other contaminants by using the ambient coastal marine area environment or a land-based aquaculture facility as the treatment process

> **depuration** means the process of reducing pathogens or other contaminants that may be present in BMS by using a managed aquatic environment as the treatment process

Relaying norovirus-contaminated shellfish to clean waters for a longer period of eight weeks is an alternative strategy to depuration, and is a method accepted by New Zealand's trading partners. Currently shellfish grown in moderately contaminated waters are required to be relayed into approved clean waters for a minimum period. The regulations for relaying are listed in Section 4, 35 of the BMS 2006 where additional requirements for virus testing before and after relaying of product are described.

"The relay period must be at least 14 consecutive days when environmental conditions are suitable for purification, but may be reduced to a minimum of 5 days, by the animal product officer, when contaminant reduction studies demonstrate that the reduced time is adequate to assure contaminant reduction.

A contaminant reduction study must be conducted by the relay operator to demonstrate the effectiveness of relaying in cleansing the shellfish of the contaminant to the background level for BMS in the relay growing area."

Depuration is usually performed in tanks. Depuration has been shown to be an inefficient means of removing viruses from shellfish (Doré *et al.,* 1998). Requirements for depuration are listed in the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 but it is not widely used in New Zealand. NZFSA has not permitted depuration as a post harvest treatment for norovirus contaminated BMS.

In New South Wales, depuration in tanks prior to sale appears to have reduced the number of oyster associated outbreaks, although this process does not remove all viruses (Fleet *et al.,* 2000). Research indicates that virus elimination by tank depuration is of low efficacy (Formiga-Cruz *et al.*, 2002; Muniain-Mujika *et al.*, 2002). Although bacteria are generally eliminated within 2-3 days, viruses are known to persist for up to 8 weeks (Greening *et al.*, 2003b; Loisy *et al.*, 2005; Nappier *et al.*, 2008). After 48 hours depuration, (Schwab *et al.,* 1998) observed a 95% reduction in bacterial numbers compared with a 7% reduction in norovirus levels in bioaccumulated shellfish. Noroviruses concentrate mainly in the digestive gland tissue but have also recently been detected in gill and other tissues (McLeod *et al.*, 2009; Wang *et al.*, 2008a). Temperature has been reported to be a significant factor in virus and bacteriophage removal, with little removal at temperatures below 9ºC (Doré *et al.*, 1998; Doré *et al.*, 2000).

5.1.3   Imported shellfish

Imported bivalve molluscan shellfish are expected to meet the same end product criteria as per the New Zealand BMS Standard. The imported food requirements for BMS are further described at: [http://www.nzfsa.govt.nz/imported-food/high-risk/bi-valve-molluscan shellfish.htm](http://www.nzfsa.govt.nz/imported-food/high-risk/bi-valve-molluscan-shellfish.htm).

BMS is permitted to be imported only from countries where the NZFSA has negotiated a pre-clearance arrangement. Pre-clearance arrangements, including the import of BMS, have been concluded with Australia, Canada, the European Community and the United States of America. For a number of other countries a pre-clearance arrangement is pending.

Pre-clearance arrangements are determined on the basis of an equivalence assessment of a country's BMS programme against the sanitary outcomes of the New Zealand production system. Consignments imported under a pre-clearance arrangement are to be monitored for a number of hazards (*E. coli*, *Listeria monocytogenes*, and marine toxins), but these do not include norovirus.

5.1.4   Controls on faecal contamination sources

Criteria for control of enteric viruses in wastewater have been generated from a quantitative microbial risk assessment (Ball *et al.*, 2008), adopted by the Northland Regional Council and incorporated into Northland wastewater treatment plant resource consents. This is particularly important for that region where there is considerable shellfish aquaculture, but as the criteria were originally derived for another site (Manukau), they may not be directly applicable as generic conditions. However, the application to other regions of New Zealand is *ad hoc* and at the discretion of local Regional Councils. In addition, the discharge consenting process may not adequately address the consequences of sewage overflows. Little monitoring of sewage for viruses occurs except at Manukau and Christchurch WWTP and therefore knowledge of the loading in sewage discharges is mostly unknown.

In 2008 the Ministry for the Environment prepared and distributed for consultation a proposed National Environmental Standard for On-site Wastewater Systems. The key proposal in the discussion document is that the owners of properties with on-site wastewater systems in specific locations will be required to hold a current warrant of fitness that confirms their on-site system is functioning properly and is being maintained to an appropriate standard. The Ministry for the Environment is currently considering the submissions, preparing a cost benefit analysis and alternative options to a national environmental standard.

5.1.5   Consumers and foodhandlers

The NZFSA has made available resources (booklets and a DVD) that promote safe food handling for seafood gatherers (http://www.nzfsa.govt.nz/consumers/hunting-collecting-fishing/seafood-gatherers/index.htm; http://www.nzfsa.govt.nz/consumers/hunting-collecting-fishing/wild-food-safety-dvd.htm).

A working group including staff from Public Health Units, the New Zealand Food Safety Authority, and ESR have developed a "Food Business Sickness Policy" and supporting material for the food industry. This is intended to be included in a Food Safety Programme and manage the risk from infected persons in food premises. It includes clearly defined minimum periods between symptoms of illness (diarrhoea or vomiting) and when the person can return to work activities that involve food handling. All foodborne pathogens are considered, including norovirus. It was recommended that food handlers infected with norovirus continue to be absent from work for a period of at least 48 hours after symptoms have ceased. This policy was trialled by several Public Health Units late in 2002, and has

now been made available to the food industry (http://www.nzfsa.govt.nz/processed-food-retail-sale/templates/sickness-template.pdf).

5.2   Options for enhanced control measures

Management of both commercial and recreational shellfish growing areas is increasingly difficult because of the failure to prevent sewage contamination of coastal and freshwater sources in shellfish growing area catchments. The impact from human recreational activities, boating, septic tank leachates, and sewage spills on shellfish growing areas requires stringent management strategies to reduce the risk of viral contamination. Nevertheless, given the limited effectiveness of depuration, and long time periods for relaying shellfish, prevention of contamination is likely to be preferable. In Northland (Waikare Inlet), the implementation of practices to improve water quality and reduce sewage discharges has shown that this can be achieved.

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7 APPENDIX 1: HAZARD AND FOOD

7.1 Growth and Survival

Noroviruses cannot grow in food. Human norovirus has not yet been grown *in vitro* although infection of an organoid cell culture was described in 2007 (Straub *et al.,* 2007). Norovirus infection of gnotobiotic calves (Souza *et al.,* 2008) and non-human primates has been achieved but there is still no representative animal model. Murine strains of norovirus are readily culturable.

7.1.1 Inactivation

Inactivation studies for norovirus have been hampered by the lack of a culture method. Dose response studies carried out in the 1970s showed that Norwalk virus retained infectivity after incubation at 60°C for 30 min (Dolin *et al.,* 1972).

Recently murine norovirus (the only known culturable norovirus) has been used as a surrogate for human norovirus in inactivation studies. These studies showed that murine norovirus was stable at low pH and sensitive to heat (63 and 72°C for 120 minutes) (Baert *et al.*, 2008; Cannon *et al.*, 2006; Hewitt and Greening, 2006).

Under refrigeration and freezing conditions the virus remains intact (and presumably viable) for several months, possibly years. Freezing generally does not inactivate viruses.

Early studies showed that Norwalk virus retained infectivity after exposure to treatment with 20% ether at 4°C for 18h and retained infectivity after exposure to pH 2.7 for 3 hr at room temperature (Dolin *et al.,* 1972). Noroviruses resist gastric acids at pH 3-4 and are able to pass through the gut intact. Norovirus has been reported to be resistant to inactivation following treatment with free residual chlorine of 0.5 to 1.0 mg/mL, but more recent studies have indicated that contamination of drinking water can be controlled by chlorination provided a sufficient contact time and concentration are achieved (Shin and Sobsey, 2008).

Noroviruses are resistant to drying. Following an outbreak in a rest home, infectious noroviruses were detected on environmental surfaces, including carpets, for over 12 days and subsequently caused more illness (Cheesbrough *et al.,* 2000).

Published data indicates that noroviruses persist in waters for extended periods (possibly weeks/months) but their infectivity status during this time is unknown. Waterborne norovirus outbreaks have been reported in many countries including New Zealand (Hewitt *et al.,* 2007).

7.2 Detection Methods

Detection of norovirus in faecal specimens from outbreaks has been carried out by the Norovirus Reference Laboratory at ESR since 1996. Development of molecular methods for typing of these viruses took place in 1997 (Meekin and Low, 1997). Additional method development provided techniques for the detection of norovirus, sapoviruses, and hepatitis A virus in shellfish (Meekin and Dawson, 1998) and other foods (Greening and Dawson, 1999).

Further development of methodology has included the use of improved primer and probe sets and the introduction of sensitive real time RT-PCR methods.

Commercial ELISA kits are now available for norovirus detection but these kits have been shown to be comparatively insensitive compared with PCR methods (Gray *et al.,* 2007), (Richards *et al.,* 2003). New Zealand kit evaluations have shown that the various kits do not provide good recognition of New Zealand strains (Greening and Hewitt, ESR, unpublished results).

Commercial RT-PCR kits for norovirus detection are not readily available. Expansion of RT-PCR capability would require the availability of standardised kits, methodologies and appropriate proficiency test programmes. The ESR Norovirus Reference Laboratory has established a biennial Australasian Proficiency Testing Programme for detection and typing of norovirus in clinical specimens. This programme evaluates and reports on the competency of 8-10 laboratories in Australia, New Zealand and Singapore. An annual proficiency testing programme or Ring Trial for norovirus and hepatitis A virus detection in shellfish samples is carried out annually by the European Community Reference Laboratory at CEFAS, Weymouth, UK. As well as the 22 EU laboratories participating, a number of non-European laboratories also participate, including the ESR Environmental and Food Virology Laboratory (EFVL). The EFVL established and validated a method for norovirus detection in shellfish which was IANZ accredited under ISO107025 in 2007. This method is now routinely used in New Zealand for viral analysis of shellfish.

The European Community and National Reference Laboratories are currently validating a standard method for norovirus detection in shellfish, which is expected to be introduced, with specific criteria for classification of production and relaying areas for bivalve molluscs, by 2012.

7.3   Overseas data on prevalence of norovirus in shellfish

The data from the scientific literature concerning overseas surveys of shellfish for norovirus are summarised in Table 4.

Table 4: Reported prevalence of norovirus in overseas raw mollusca

Country	Mollusc species	Samples tested	Positive for NOROVIRUS (%)	Year	Reference
France	Oysters	108	23	1995-1998	(Le Guyader *et al.,* 2000)
France	Mussels	73	35.6	1995-1998	(Le Guyader *et al.,* 2000)
Greece	Oysters, mussels	144	1.4	2000-2001	(Formiga-Cruz *et al.,* 2002)
Hong Kong	Oysters (imported from 11 countries worldwi	507	10.5	2000-2002	(Cheng *et al.*, 2005)

Country	Mollusc species	Samples tested	Positive for NOROVIRUS (%)	Year	Reference
	de)				
Italy	Oysters, mussels, clams	235	14 (after depuration)	NS	(Croci *et al.*, 2007)
Italy	Mussels, clams, oysters	137	2.1	2006	(Gabrieli *et al.*, 2007)
Japan	Clams	57	54	2005-2006	(Hansman *et al.*, 2008)
The Netherlands	Oysters	21	4.8	2003-4	(Boxman *et al.*, 2006)
The Netherlands	Oysters	64	0	2001	(Lodder and de Roda Husman, 2005)
Norway	Mussels & oysters	681	6.8	2000-3	(Myrmel *et al.*, 2004)
Spain	Oysters, mussels	104	25	2000-2001	(Formiga-Cruz *et al.,* 2002)
Sweden	Oysters, mussels	54	76	2000-2001	(Formiga-Cruz *et al.,* 2002)
Tunisia	Mussels, clams	23	35	2000-2001	(Elamri *et al.*, 2006)
UK	Oysters	32	56 (prior to depuration)	1995-6	(Henshilwood *et al.,* 1998)
UK	Oysters	32	38 (after depuration)	1995-6	(Henshilwood *et al.,* 1998)
UK	Oysters	32	73 (summer)	1995-6	(Henshilwood *et al.,* 1998)
UK	Oysters	32	31 (winter)	1995-6	(Henshilwood *et al.,* 1998)
UK	Oysters	3 (site 1 summer)	0	1995-1997	(Doré *et al.*, 2000)
UK	Oysters	4 (site 1 winter)	0	1995-1997	(Doré *et al.*, 2000)
UK	Oysters	5 (site 2 summer)	0	1995-1997	(Doré *et al.*, 2000)*
UK	Oysters	10 (site 2 winter)	0	1995-1997	(Doré *et al.*, 2000)*
UK	Oysters	6 (site 3 summer)	0	1995-1997	(Doré *et al.*, 2000)*
UK	Oysters	7 (site 3 winter)	0	1995-1997	(Doré *et al.*, 2000)*
UK	Oysters	14 (site 4 summer)	0	1995-1997	(Doré *et al.*, 2000)*
UK	Oysters	21 (site 4	62	1995-	(Doré *et al.*, 2000)*

Country	Mollusc species	Samples tested	Positive for NOROVIRUS (%)	Year	Reference
		winter)		1997	
UK	Oysters, mussels	173	5.8	2000-2001	(Formiga-Cruz *et al.,* 2002)
UK	Oysters (commercial)	237	59	2004-2006	(Lowther *et al.,* 2008)
USA	Oysters	45 (sampling sites)	44	2002-2003	(Costantini *et al.*, 2006)

*These oysters were depurated for 42hr pre-analysis

8 APPENDIX 2: EVALUATION OF ADVERSE HEALTH EFFECTS OVERSEAS

The 1993-1996 Infectious Intestinal Disease (IID) Study in England (Wheeler *et al.,* 1999) reported that of the estimated 9.4 million cases of illness each year (approximately 1 in 5 people each year), 1.5 million cases (17%) presented to their general practitioner. The IID study failed to detect an enteric pathogen or toxin in 49% of cases of gastroenteritis. In a follow-up study (Amar *et al.,* 2007), polymerase chain reaction assays for the detection of a range of enteric pathogens were applied to archived samples from the case-control arm of the study. The percentage of archived samples from cases and controls in which at least one pathogen or toxin was detected increased from 53% in the original study to 75%, and from 19% to 42%, respectively. The greatest change was in the detection of viruses, with *C. jejuni* dropping from being the most commonly identified pathogen, to being third after norovirus and rotavirus A. Amongst cases, norovirus and rotavirus were detected in 36% and 31% of faecal samples respectively. These results suggest that approximately 70% of all cases of infectious intestinal disease in the United Kingdom are caused by viruses (norovirus, rotavirus and sapovirus).

A study in the United Kingdom (Lowther *et al.,* 2008) of norovirus contamination in oysters from commercial harvesting areas found that contamination peaked during winter months (northern hemisphere October – March) which was consistent with epidemiological data showing higher levels of shellfish-associated norovirus infection in winter. The authors postulated that there may be a positive feedback relationship between higher prevalence in the human population and seeding into the marine environment during winter. No seasonal prevalence in human norovirus disease has been observed in New Zealand.

Representative reports from the scientific literature concerning outbreaks of norovirus overseas are summarised in Table 5.

Table 5: Examples of norovirus outbreaks associated with raw mollusc consumption overseas

Country	Food implicated	No. ill	Attack rate (%)	Evidence for food implicated	Reference
Australia	Oysters	97	NS	Epidemiological	(Stafford *et al.,* 1997)
Australia	Oysters (raw)	83	17 & 35	Epidemiological, RT-PCR of oysters and faeces	(Webby *et al.,* 2007)
Australia	Oysters (raw)	19	56	Epidemiological, traceback, immunoassay of faecal sample from one case	(Huppatz *et al.,* 2008)
Canada	Oysters (raw)	79	NS	Epidemiological, RT-PCR of oysters and faeces	(David *et al.,* 2007)
France	Oysters	205	NS	Epidemiological, RT-PCR of oysters and faeces detected multiple enteric viruses	(Le Guyader *et al.,* 2008)

France	French oysters (raw)	127	NS	Epidemiological, RT-PCR of oysters and faeces	(Le Guyader *et al.*, 2006b)
Italy		202	92-100		
Italy	Mussels (raw)	103	74	Epidemiological, RT-PCR of mussels and faeces	(Prato *et al.*, 2004)
Singapore	Oysters (raw)	305	>82	Epidemiological, RT-PCR on faecal samples, electron microscopy on oyster samples	(Ng *et al.*, 2005)
UK	Oysters (raw)	15	100	Epidemiological, RT-PCR of faeces	(Gallimore *et al.*, 2005)
UK	Oysters	9	38%	Epidemiological, examination of stools	(Ang, 1998)
USA	Oysters, steamed and raw	>180	43-100%	Epidemiological, electron microscopy of stools	(FDA, 1993)
USA	Oysters	45	63%	Epidemiological, raised antisera, electron microscopy of stools	(FDA, 1994)
USA	Oysters	70	83%	Epidemiological, electron microscope and RT-PCR examination of stools.	(Kohn *et al.,* 1995)
USA	Oysters	171	NS	Epidemiological, RT-PCR of viruses from oysters	(Shieh *et al.,* 2000)

NS = not stated

9 APPENDIX 3: EVALUATION OF RISK

9.1 Settings for norovirus outbreaks in New Zealand

The Norovirus Reference Laboratory, in addition to analysing faecal specimens, consolidates information on outbreaks which can provide a more detailed picture of the epidemiology. This enables a larger number of outbreaks to be identified, and outbreaks initially reported as gastroenteritis can be assigned as norovirus**Error! Reference source not found.** shows the settings for the 935 norovirus outbreaks from 2001-2007. The importance of healthcare settings is clear, as is the large number of outbreaks assigned as catered events (these settings include restaurants, cafes, takeaway bars as well as catered events). These data highlight the importance of food-handlers and person-to-person transmission.

Figure 3: Settings for Norovirus outbreaks 2001-2007 (n= 935) from data assembled by the Norovirus Reference Laboratory