Food Microbiology 69 (2018) 196–203

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

National survey of foodborne viruses in Australian oysters at production

Valeria Torok [a, *], Kate Hodgson [a], Catherine McLeod [a, b], Jessica Tan [a], Navreet Malhi [a], Alison Turnbull [a]

[a] South Australian Research and Development Institute, Food Safety and Innovation, GPO Box 397, Adelaide, South Australia, 5001, Australia
[b] Seafood Safety Assessment Ltd. Hillcrest, Kilmore, Isle of Skye, IV44 8RG, United Kingdom

ARTICLE INFO

Article history:
Received 19 January 2017
Received in revised form
15 August 2017
Accepted 18 August 2017
Available online 22 August 2017

Keywords:
Norovirus
Hepatitis A virus
Crassostrea gigas
Saccostrea glomerata

ABSTRACT

Internationally human enteric viruses, such as norovirus (NoV) and hepatitis A virus (HAV), are frequently associated with shellfish related foodborne disease outbreaks, and it has been suggested that acceptable NoV limits based on end-point testing be established for this high risk food group. Currently, shellfish safety is generally managed through the use of indicators of faecal contamination. Between July 2014 and August 2015, a national prevalence survey for NoV and HAV was done in Australian oysters suitable for harvest. Two sampling rounds were undertaken to determine baseline levels of these viruses. Commercial Australian growing areas, represented by 33 oyster production regions in New South Wales, South Australia, Tasmania and Queensland, were included in the survey. A total of 149 and 148 samples were collected during round one and two of sampling, respectively, and tested for NoV and HAV by quantitative RT-PCR. NoV and HAV were not detected in oysters collected in either sampling round, indicating an estimated prevalence for these viruses in Australian oysters of <2% with a 95% confidence interval based on the survey design. The low estimated prevalence of foodborne viruses in Australian oysters was consistent with epidemiological evidence, with no oyster-related foodborne viral illness reported during the survey period.

© 2017 Elsevier Ltd. All rights reserved.

1. Introduction

Human enteric viruses are increasingly recognised as important causes of foodborne disease globally, based on the incidence of reported foodborne disease and the severity of disease (including mortality) (FAO/WHO, 2008, 2012). International estimates of the proportion of enteric virus illnesses attributed to food are in the range of approximately 5% for hepatitis A virus (HAV) and 12–47% for norovirus (NoV). The virus-commodity combinations of greatest public health concern are NoV and HAV in bivalve molluscs, fresh produce and prepared (ready-to-eat) foods (FAO/WHO, 2008, 2012). A systematic review of global shellfish related viral foodborne outbreaks between 1980 and 2012 reported NoV (83.7%) and HAV (12.8%) as the most common viral pathogens and oysters (58.4%) as the most frequently consumed shellfish associated with outbreaks (Bellou et al., 2013). The majority of the reported outbreaks have been located in East Asia, followed by Europe, America, Oceania, Australia and Africa (Bellou et al., 2013). In Australia, between 2001 and 2010, seventeen suspected foodborne outbreaks of NoV or unknown aetiology were associated with consumption of bivalve shellfish, which included imported product (OzFoodNet Reports). A recent oyster related outbreak of NoV occurred in 2013 with 525 people affected nationally following consumption of contaminated oysters from Tasmania (Lodo et al., 2014).

As there are currently no effective control measures available to eliminate these viruses from food without changing the characteristics of the product, the most effective risk management strategy for NoV and HAV in bivalve shellfish is to prevent contamination in production areas. Freezing of shellfish does not deactivate foodborne viruses, but rather preserves them (EFSA, 2012). High-risk factors for contamination of oysters with enteric viruses include low water temperatures (allowing greater persistence of the viruses), elevated prevalence of enteric illness within the community and high rainfall leading to sewage system overflows (CEFAS, 2011).

* Corresponding author.
E-mail address: valeria.torok@sa.gov.au (V. Torok).

http://dx.doi.org/10.1016/j.fm.2017.08.014
0740-0020/© 2017 Elsevier Ltd. All rights reserved.

In 2012, the Codex Alimentarius commission released guidelines on general principles of food hygiene to control viruses in food, with Annex I specifically focusing on control of HAV and NoV in bivalve molluscs (FAO/WHO, 2008). It recommended that countries monitor for NoV and HAV in bivalves following shellfish-related foodborne outbreaks and high-risk pollution events (heavy rainfall and overflow from sewage treatment plants). The EU legislation on the microbiological criteria for foodstuff has suggested that "criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently" (EC, 2005). With the development of the ISO/TS 15216 method "Microbiology of food and animal feed - horizontal method for the determination of hepatitis A virus and norovirus in food using real-time RT-PCR" (ISO/CEN, 2013), virus methods have become available that may be considered suitable for use in legislation. Hence, consideration is being given to establishing virus limits for high-risk live bivalve molluscs. The EFSA Scientific Opinion on NoV in oysters recommended: the establishment of an acceptable limit for NoV in oysters to be harvested and placed on the market; NoV testing of oysters to verify compliance with the acceptable NoV limits established; and for food businesses to verify their Hazard Analysis and Critical Control Points plans and demonstrate compliance with acceptable levels (EFSA, 2012). In 2012, the EU Community Reference Laboratory recommended that if virus standards are introduced, then standards for NoV should be quantitative (i.e. a maximum acceptable level) and standards for HAV be qualitative (i.e. presence/absence) (CEFAS, 2013). It also considered and made recommendations on possible levels for a NoV standard in the context of both end-product and production area monitoring applications (CEFAS, 2013). The EU is current undertaking a two year survey to establish European prevalence of NoV contaminated oysters at the production area and dispatch centre levels (EFSA, 2016). Following this survey, the European Commission will appraise the results and decide whether microbiological criteria for NoV are appropriate.

The prevalence of NoV in oysters internationally has been reported to range from 2.4% to 76.2% (Lowther et al., 2012; Pavoni et al., 2013; Suffredini et al., 2014). Information on the prevalence of NoV in Australian oysters is limited, but suggests a low prevalence. A study of oysters from growing areas at risk of contamination, over a range of environmental conditions, found NoV in 1.7% of oysters sampled (Brake et al., 2014). As a response to the impending international regulations (noting that some nations already require NoV testing on imported products e.g. Singapore), the Australian oyster industry desired a more comprehensive evaluation of the prevalence of enteric foodborne viruses in Australian oysters at production. Similar surveys have been undertaken worldwide, and have found that the prevalence of foodborne viruses in oysters obtained in market products were comparable to those observed in commercial harvesting areas (EFSA, 2012). The current study aimed to estimate the national prevalence of NoV and HAV in Australian oysters suitable for harvest. The survey used the ISO/TS 15216 standard testing methodology for foodborne viruses in shellfish and a robust statistical sampling plan conducted over two rounds of sampling between July 2014 and August 2015.

2. Material and methods

2.1. Survey design

The design called for a total of 300 oyster samples to be collected over 13 months between July 2014 and August 2015 in two sampling periods, representing a winter/spring (round 1) and a summer/autumn (round 2) period. A sample size of 150 for each of the two sampling rounds would provide a statistical probability of 0.95 of detecting at least one sample with detectable levels of viruses if ≥2% of the samples were contaminated. The sample size calculation was based on the binomial distribution:

$$P(X = \mathrm{x}) = \left(\frac{n}{x} p^{\mathrm{x}} (1 - P)^{n-\mathrm{x}}\right)$$

where X is the discrete random variable representing the number of samples with detected virus out of the total number of samples, x = 0, p = 0.02 (assumed prevalence) and n, the total sample size, is the variable of interest. In addition, the largest margin of error for a prevalence estimate with this sample size is ±8% (for a 95% confidence interval).

Oyster samples were collected from all major oyster harvest areas within Australia, including the states of New South Wales (NSW), South Australia (SA), Tasmania (Tas) and Queensland (Qld). Pacific oysters (*Crassostrea gigas*) were sampled in SA, Tas and NSW, whereas Sydney rock oysters (*Saccostrea glomerata*) were sampled in NSW and Qld. The total number of samples to be collected per state was informed by five years of national oyster production data from 2007–08 to 2011–12, obtained from the ABARES Australian Fisheries and Aquaculture Statistics for edible oysters (ABARES, 2012). Sampling plans and assignment of sample numbers to production areas within each state were based on state production data over a five year period, with the exception of SA, where only data for a three year period (2008–12) were available. Data for NSW were obtained from NSW Aquaculture Production Reports (http://www.dpi.nsw.gov.au/fisheries/aquaculture/publications/aquaculture-production-reports). Data for SA were obtained from Primary Industries and Regions South Australia, Aquaculture Policy and Planning Programs. Data for Tas were obtained from Department of Primary Industries, Parks, Water and Environment, Marine Resources. In Qld oyster production is limited to Moreton Bay and data were obtained from Aquaculture Policy and Industry Development, Fisheries Queensland. Further information on oyster production, broken down to harvest areas, was provided by each state's industry and regulatory bodies. The proportional production per harvesting area was used to weight the probability of assigning a sample to a particular harvest area in a randomised manner. Within each harvesting area, the particular oyster lease for sample collection was determined by unweighted randomised sampling based on active leases producing mature oysters. Samples were randomly allocated to the identified harvest areas in fortnightly blocks. The final sampling schedule and all associated steps were determined using R software (R Core Development Team, version 3.1.3) to avoid any bias. Samples were only collected from leases that were considered by regulators to be fit for human consumption from an enteric virus perspective. This includes *Approved* and *Conditionally Approved* growing areas in the open status, (same classification in the US National Shellfish Sanitation Program and equivalent to Class A waters in the EU), or shellfish suitable for depuration from *Conditionally Approved* or *Restricted* areas in NSW only (approximately equivalent to Class B waters) (ASQAAC, 2016; EC, 2004; FDA, 2015). Sampling kits were provided with cooling pads and instructions. On receipt, the condition of the samples was checked and logged, and samples stored at −80 °C until testing. Samples were generally received within 2 days of being sent. Of the samples not sent frozen (n = 276) the arrival temperature was 13.7 °C (average), 15.1 °C (median).

2.2. Analytical testing for foodborne viruses

The method used for testing for NoV genotype I (GI), NoV genotype II (GII) and HAV in oysters was as outlined within the IOS/TS 15216 method "Microbiology of food and animal feed – horizontal

method for determination of HAV and NoV in food using real-time RT-PCR" with the exception that murine norovirus (MNV-1) was used instead of Mengo virus as the process control virus (ISO/CEN, 2013).

Oysters were thawed overnight at 4 °C prior to sample preparation. Samples were comprised of 12 individual oysters collected from one lease. Oysters were scrubbed under potable running water prior to being shucked and the oyster meat collected into clean press seal plastic bags. The digestive tissue (DT) from each individual oyster within a sample was dissected out with scalpels, transferred to a clean petri dish and finely chopped to produce a composite sample. A 2 g sub-sample of the DT was taken and 10 μl (6.6×10^4 plaque forming units/ml) of the process control virus (MNV-1) and 2 ml of Proteinase K solution (3 units/ml; Astral Scientific, Australia) were added to the sample. Samples were mixed and incubated at 37 °C, shaking (320 rpm) for 60 min. Any residual untreated DT was stored at −20 °C for re-testing if required. Following the initial incubation, samples were transferred to a water bath and incubated at 60 °C for 15 min without shaking. Following the second incubation, samples were centrifuged at $3000 \times g$ for 5 min and the supernatant recovered; the volume was recorded and retained at −20 °C for downstream nucleic acid extraction.

Extraction and purification of viral RNA was done using the bioMerieux NucliSENS® Minimag system (bioMerieux Pty. Ltd. Baulkham Hills, NSW, Australia), following the manufacturer's recommendations. In brief, RNA was extracted from the entire virus using guanidine isothiocyanate which disrupts the viral coat protein. The viral RNA was adsorbed onto magnetic silica beads, washed with various buffers and released into 100 μl of elution buffer. Each batch of nucleic acid extractions included a negative extract control (sterile water), as well as in-house positive control (a 10 μl aliquot of the process control virus, MNV-1).

Real-time RT-PCR for HAV, NoV GI and NoV GII was done using primers and probes as specified in ISO/TS 15216-1 (ISO/CEN, 2013). Primers and probes for real-time RT-PCR of the process control virus (MNV-1) were those specified by Hewitt et al. (2009) (Hewitt et al., 2009).

The RT-PCR master mix used for all assays was the RNA Ultrasense™ one-step qRT-PCR system (Life Technologies, Mulgrave, Vic, Australia), prepared following the manufacturer's recommendations. Real-time RT-PCR cycling parameters were as specified in ISO/TS 15216-1 and included an initial incubation at 55 °C for 1 h followed by denaturation at 95 °C for 5 min and 45 cycles of 95 °C for 15 s, 60 °C for 1 min and 65 °C for 1 min. Real-time RT-PCR was run in a 384-well format (ViiA™ 7 system, Applied Biosystems, Mulgrave, Vic, Australia), with master mix and template being dispensed using a Biomek 3000 Laboratory Automation Workstation (Beckman Coulter, Fullerton, CA, USA). The ISO/TS 15216 protocol outlines a number of controls and standards including the use of a process control virus (MNV-1) to determine virus extraction efficiency and External Control (EC) RNA to determine amplification efficiency.

2.3. Proficiency testing for detection of foodborne viruses in bivalve molluscs

South Australian Research and Development Institute (SARDI) Food Safety and Innovation has participated in the European Reference Laboratory Proficiency (EURL) testing scheme for shellfish organised by the Centre for Environment, Fisheries and Aquaculture Science, UK (CEFAS-UK) in 2012 (PT 46) and 2013 (PT 50). On each occasion, these trials involved quantitative analysis of four contaminated shellfish samples and two lenticule discs (reference material) for HAV, NoV GI and NoV GII using the laboratory's method of choice. The testing methodology was as detailed in ISO/TS 15216-1 for bivalve molluscan shellfish and SARDI Food Safety and Innovation laboratories scored 100% in relative accuracy, specificity and sensitivity in both of these trials.

2.4. Statistical analysis

The R software (R Core Development Team, version 3.1.3) was used to perform statistical analysis and generate the prevalence estimates, including associated 95% confidence intervals for NoV GI, NoV GII and HAV. The estimate of prevalence is the number of samples with detected levels of virus expressed as a proportion of the total number of samples. In the instance of no detections, it can be concluded that the estimated prevalence is low, <2% (with 95% confidence), based on the assumptions of the sample size calculation. The upper bound for the prevalence estimate was calculated based on the sample size and a 95% probability of detecting at least one sample with detectable levels of viruses.

3. Results

3.1. National oyster sampling

Between 2007–08 and 2011–12, the annual average production of oysters per state was valued at AU $40.7 million, AU $0.5 million, AU $34.6 million and AU $21.8 million for NSW, Qld, SA and Tas, respectively (ABARES, 2012). Production in other Australian states is insignificant by value and not captured in the statistics. The proportion of state oyster production to the overall national annual production (by value) was used to allocate sample numbers for collection from each state. For NSW, 63 samples were collected in the first sampling round and 62 in the second sampling round to give a total of 125 samples for the overall survey. In Tas, 33 and 34 samples were collected in sampling rounds 1 and 2 respectively. In SA, 53 samples were collected in each round and in Qld one sample was collected in each round. The proportion of oyster production from growing areas within states and the sample allocation per planned sampling period are shown in Table 1, along with growing area classifications. Locations and numbers of samples to be collected from each production area nationally during each of the two sampling periods are shown in Fig. 1.

The actual number of samples collected per sampling round did not vary from the original sampling plan in SA or Qld (Table 1). In Tas, the sampling plan varied in that the one sample to be collected from the Far North West production area in sampling round one could not be collected in the designated timeframe due to closures and instead was included in sampling round two.

The actual sample numbers received per sampling round from NSW are shown in Fig. 2. Variation in sample numbers between seasons was due to the need to reallocate samples if a harvest area was closed, the growing area was unwilling to participate in the survey, or the requested samples were not submitted. Only one growing area was unwilling to participate, whilst another production area did not submit samples for the survey. A total of three samples from these areas were to be collected in each sampling round. These samples were statistically reallocated to other production areas within NSW using the randomised procedures detailed above.

Numerous weather induced harvest area closures occurred during each of the sampling rounds, hence sample collection was often delayed and in some cases needed to be reallocated. As a

Table 1
National sampling plan for oysters collected from production areas.

State/Growing area classification	Production area	Proportion production to state (%)	Seasonal sample numbers
New South Wales			
Conditionally Restricted	Nambucca River	1.9	1
Conditionally Approved & Conditionally Restricted	Macleay River	0.9	1
Conditionally Approved & Conditionally Restricted	Hastings River	4.1	2
Conditionally Approved & Conditionally Restricted	Camden Haven	3.1	2
Conditionally Restricted	Manning River	1.5	1
Conditionally Approved	Wallis Lakes	35.4	22
Conditionally Approved & Conditionally Restricted	Port Stephens	15.9	10
Conditionally Restricted	Brisbane Water	3.9	2
Conditionally Approved	Hawkesbury River	0.1	1
Conditionally Approved & Conditionally Restricted	Shoalhaven - Crookhaven River	2.5	2
Conditionally Approved	Clyde River	11.9	7
Conditionally Approved	Turross Lake	0.8	1
Conditionally Approved	Wagonga Inlet	4.0	2
Conditionally Approved	Wapengo Lake	1.1	1
Conditionally Approved	Merimbula Lake	3.8	2
Conditionally Approved	Pambula River	3.6	2
Conditionally Approved	Wonboyn River	1.1	1
Conditionally Approved & Conditionally Restricted	Other [a]	4.3	3
South Australia			
Approved & Conditionally Approved	Coffin Bay	60.2	31
Approved	Smoky Bay	19.4	10
Approved	Cowell	9.7	5
Approved	Streaky Bay	5.5	3
Approved	Ceduna (Denial Bay)	4.2	2
Approved	Kangaroo Island (Nepean Bay)	0.7	1
Approved	Yorke Peninsula (Central Yorke)	0.3	1
Tasmania			
Approved & Conditionally Approved	D'Entrecasteaux Channel	8.6	3
Conditionally Approved	Far North West	6.6	2
Conditionally Approved	Georges Bay	17.8	6
Conditionally Approved	Great Oyster Bay	6.5	2
Approved & Conditionally Approved	Tasman Peninsula	16.9	6
Conditionally Approved	Pipe Clay Lagoon	27.2	9
Conditionally Approved	Pittwater	16.4	6
Queensland			
Conditionally Approved	Moreton Bay	100	1

[a] Other growing areas included Tweed, Richmond, Clarence, Wooli Wooli, Belinger and Bermagui Rivers and Nelson Lagoon.

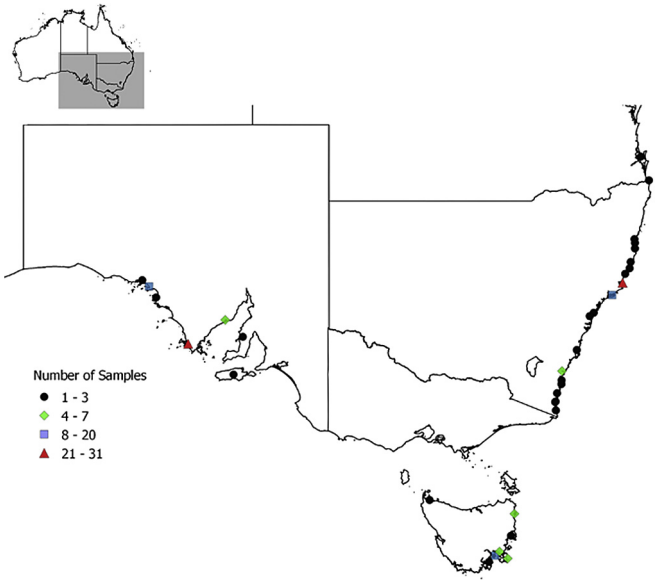

Fig. 1. Locations and numbers of oyster samples collected during each sampling round.

result, sampling for round one was from July 2014 through to November 2014, instead of the planned September 2014. Sampling for round two was from January 2015 through to August 2015, instead of the planned March 2015. Fig. 3 shows actual sample numbers received per month from each state during the survey.

3.2. Prevalence estimates for foodborne viruses in Australian oysters at production

A total of four samples showed unacceptable virus extraction efficiency (<1%) and were excluded from calculation of virus prevalence estimates. Of the remaining samples included in the calculation of the prevalence estimate, 25 samples showed a virus extraction efficiency of ≤10% and 268 had a virus extraction efficiency of >10%. The amplification efficiencies were used for quality assurance and not to adjust the results. Unacceptable amplification efficiencies (<25%) were obtained for 18 NoV GI, 14 NoV GII and 4 HAV test results. Of these, 12 test samples showed an unacceptable amplification efficiency for NoV GI and GII, one for NoV GI and HAV only, and three for all three viruses. When a test sample resulted in unacceptable amplification efficiency, the test was deemed invalid and the sample excluded from the calculation of the prevalence estimate.

Norovirus (NoV GI or NoV GII) was not detected in oysters during either round of sampling. Of the 149 oyster samples received and analysed during round one of the survey, 141 and 142 samples gave valid test results for NoV GI and GII, respectively. During round

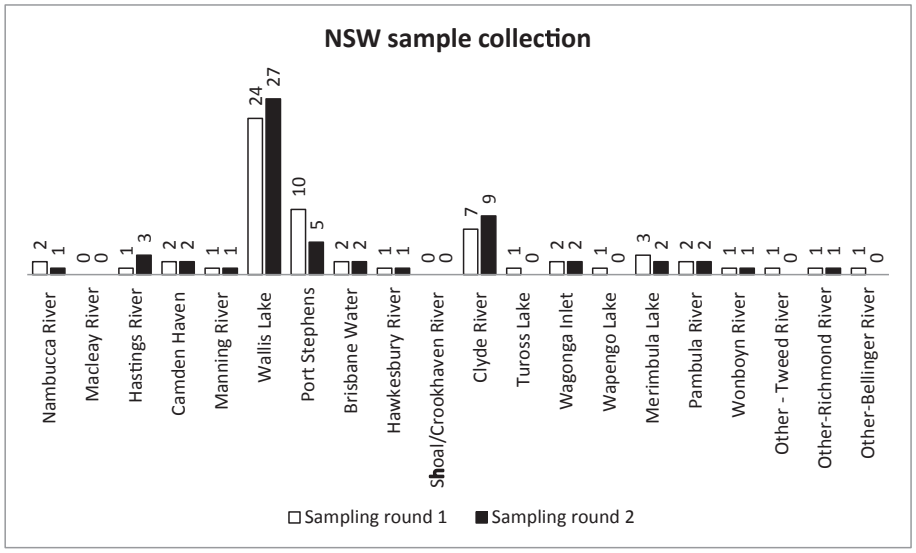

Fig. 2. Actual sample numbers collected from NSW oyster production areas per sampling round.

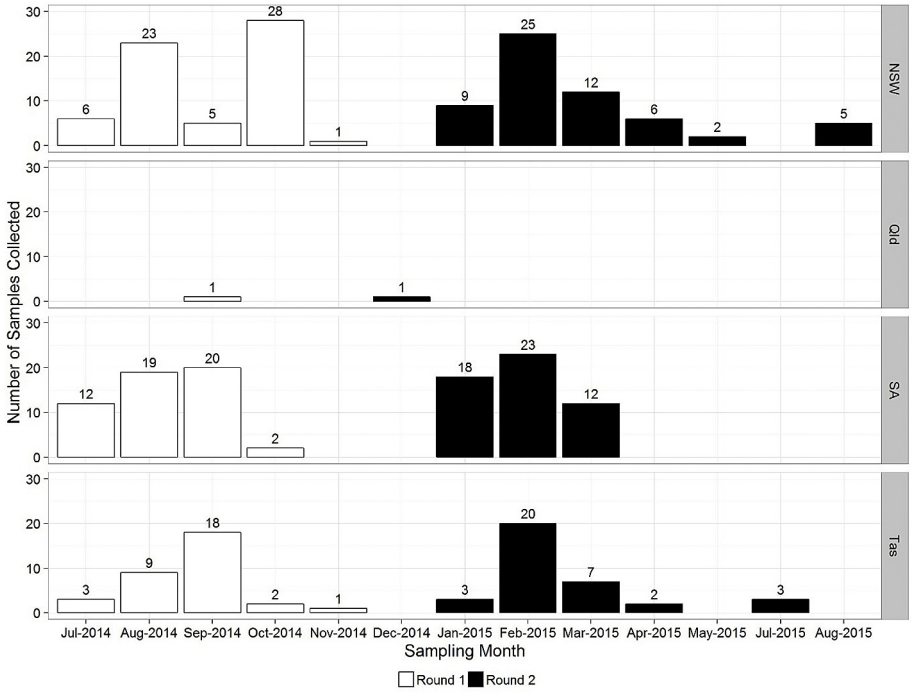

Fig. 3. Sample numbers received per month during rounds one and two of the survey.

two of the survey, 148 samples were received and analysed, of which 134 and 137 gave valid test results for NoV GI and GII, respectively. HAV was not detected in oysters during either round of sampling. Of the 149 oyster samples received and analysed in the first round of sampling, 147 gave valid test results. Of the 148 samples received and analysed in the second round of sampling, 142 gave valid test results. Based on the results of the survey for NoV and HAV in Australian oysters at production during 2014–15, the prevalence estimate for these viruses is <2% in both rounds of sampling (Table 2).

4. Discussion

This is the first Australian survey of foodborne viruses in commercially produced oysters where the sampling plan has been statistically designed to estimate prevalence based on production by region. This survey only sampled mature shellfish taken from harvest areas which were open for harvest. For Tas, SA and Qld this represented oysters that were considered to be fit for direct harvest and consumption. For NSW, this represented either oysters fit for direct harvest and consumption, or fit for depuration prior to

Table 2
Prevalence estimates for NoV and HAV in Australian oysters at production.

Virus	Prevalence estimate (95% confidence interval)	Number of virus detections	Number of valid test results
Sampling round 1			
NoV GI	<2% (0–2.6%)	0	141
NoV GII	<2% (0–2.6%)	0	142
HAV	<2% (0–2.5%)	0	147
Sampling round 2			
NoV GI	<2% (0–2.7%)	0	134
NoV GII	<2% (0–2.7%)	0	137
HAV	<2% (0–2.6%)	0	142

market. Two sampling rounds were chosen over a year to enable detection of seasonal variation in virus prevalence. In total, 33 national production regions were asked to participate in the survey, of which only two did not provide samples. Both of these areas were minor NSW production areas supplying in total 1.4% of national production. During the 2014-15 survey, NoV and HAV were not detected in oysters during either sampling round, which translated into an estimated prevalence of <2% (95% CI 0–2.7%) for these viruses in Australian oysters suitable for harvest.

In Australia, it has been estimated that the cost of all foodborne gastroenteritis (bacterial and viral pathogens in all food types) is AU $811 million annually (Abelson et al., 2006). This is largely due to losses in productivity, lifestyle and premature mortality. Costs, however, to health care services, surveillance programs, primary producers, food industries and trade are also significant. The cost attributed to NoV and specifically to oysters is not available. However, one widely reported outbreak of hepatitis A in 1997 caused by consumption of contaminated oysters from Wallis Lake, NSW, was responsible for an estimated 444 cases of illness and one death (Conaty et al., 2000). This outbreak had a multifactorial negative economic impact which included a national health cost of disease estimated at AU $12.1 million and a 15–20% decrease in the market share for the local oyster industry (net income loss of AU $500,000 per annum in 1997 and the few following years). Other broader negative impacts were estimated losses of AU $0.2 and AU $1.0 million to the local fishing and accommodation sectors respectively in 1997, and reduced public confidence in oysters (Handmer and Hillman, 2004; OzFoodNet, 2006). As a result of the Wallis Lake incident and several other NoV outbreaks, NSW significantly changed the risk management of oyster growing areas, in line with the guidelines in the Australian Shellfish Quality Assurance Program (ASQAP) Manual of Operations (ASQAAC, 2016).

Risk management for bivalve shellfish currently relies on the use of enteric bacteria as indicators of faecal contamination. Shellfish quality assurance programs world-wide classify production areas for shellfish harvest as fit for human consumption, requiring depuration, or prohibited for harvest based on sanitary surveys of the catchment, including levels of bacterial indicator organisms in shellfish tissues or in waters where shellfish are grown, in accordance with Section 7 of the Codex Alimentarius code of practice for fish and fishery products (FAO/WHO, 2003). Although, coliforms and *E. coli* are good indicators of recent faecal contamination of growing waters by warm blooded animals, they are not good indicators of the presence of human enteric viruses. Flannery et al. (2009) reported that 31% of compliant oysters harvested from Class A Irish waters were positive for NoV between 2005 and 2007. On an individual sample basis, *E. coli* is a poor predictor of NoV risk, although on a site-specific basis average *E. coli* levels have been shown to correlate with average NoV levels when UK winter period data were investigated (Lowther et al., 2012). Enteric viruses are more resistant to wastewater treatment and are more environmentally stable than faecal bacterial indicators (Da Silva et al., 2007). Viruses are also concentrated at higher levels and persist longer in shellfish than bacteria (EFSA, 2012). Depuration is effective in purging shellfish of bacterial contamination but ineffective in significantly reducing viral contamination (McLeod et al., 2017; Muniain-Mujika et al., 2002; Polo et al., 2014).

Over the past decade several international studies have investigated the presence of human enteric viruses, including NoV and HAV, in commercial and wild bivalve shellfish (oysters, mussels, clams and cockles) from Australia, France, Ireland, Italy, Japan, Korea, Morocco, Spain, UK and USA (Benabbes et al., 2013; Brake et al., 2014; Costantini et al., 2006; DePaola et al., 2010; Doré et al., 2010; Flannery et al., 2009; Loutreul et al., 2014; Lowther et al., 2012; Maekawa et al., 2007; Moon et al., 2011; Pavoni et al., 2013; Polo et al., 2015; Schaeffer et al., 2013; Seo et al., 2014; Shin et al., 2013; Suffredini et al., 2014). Comparison of the various published studies is difficult for a variety of reasons including: use of varied sample preparation and detection methodologies; surveys not being representative of a country's national production; not discriminating for oysters which are deemed fit for human consumption as raw product; absence of statistically valid sampling plans; and often point-in-time investigations. The current survey for NoV in Australian oysters is one of only a few internationally that has investigated national production comprehensively (DePaola et al., 2010; Lowther et al., 2012; Pavoni et al., 2013). It is only the second known study of HAV testing in oysters at a national level, with the other study occurring in the USA (DePaola et al., 2010).

The presence of NoV in oysters has been reported in the range of 3.9–20% in the USA (Costantini et al., 2006; DePaola et al., 2010), 14.1–45% in Korea (Moon et al., 2011; Seo et al., 2014; Shin et al., 2013), 4.2% in Japan (Maekawa et al., 2007), 2.4% (Class A harvest area) and 47.8% (Class B harvest area) in Italy (Pavoni et al., 2013; Suffredini et al., 2014), 9% in France (Schaeffer et al., 2013), 2.9% in Morocco (Benabbes et al., 2013), 76.2% in the UK (Lowther et al., 2012) and 31% (Class A harvest area) in Ireland (Flannery et al., 2009). Less information is available on HAV in oysters, but the presence is generally much lower, with 4.4% reported in the USA (DePaola et al., 2010) and none detected in Korean oysters (Seo et al., 2014). HAV has been reported in Spanish shellfish (mussels, cockles and clams) from Class B harvesting areas with a prevalence of 10.1% (Polo et al., 2015). These studies have provided information on the level of contamination in shellfish, however, none have related results back to epidemiological data as an indicator for the risk of shellfish-related illness.

The only other study of NoV in Australian oysters available, investigated limited geographic sites (n = 6) in NSW, SA and Tas (two sites per state). In order to provide a worst case estimate of viral contamination of Australian oysters, the survey purposefully targeted sites that were considered most compromised with respect to the potential for human faecal contamination, and did not discriminate amongst harvest site classification or harvest status (Brake et al., 2014). NoV was detected in 1.7% of sampled

oysters (2/120), however these samples were taken from growing areas closed to harvest and, therefore, were not representative of viral levels in market oysters. The previous study supports our observation of a very low prevalence of human enteric viruses (<2%) in Australian oysters. In the current study a significantly larger number of collected samples per round would have been required to increase the accuracy in this estimate of the prevalence below the upper limit of 2%. In contrast, oysters harvested from European Class A waters, approximately equivalent to the Australian open for harvest classification, have reported varied levels of NoV, ranging from 2.4% in Italy (Pavoni et al., 2013) to 76.2% in the UK (Lowther et al., 2012).

Virus extraction and detection methodologies reported in prior studies have been varied among and even within investigations (Pavoni et al., 2013). Many studies have failed to use adequate controls for virus extraction and amplification efficiency. Virus extraction methodology can have a major impact on the recovery of foodborne viruses from the oyster matrix and in the ISO/TS 15216 method it is recommended that an exogenous process control virus be used to determine virus recovery (ISO/CEN, 2013). Detection methodology (end-point RT-PCR, quantitative RT-PCR, primers and probes) can impact on sensitivity and specificity of the assay. The publication of the ISO/TS 15216 method for the detection of NoV and HAV in foodstuffs, including bivalve shellfish, will allow results from future studies employing this methodology to be more comparable amongst investigations. Although this method does not distinguish between infective and non-infective virus, it does enable both the determination of virus prevalence and quantification of viral RNA, and provides a basis for participation in internationally run proficiency trials.

The majority of investigations into NoV and HAV in bivalve shellfish have been undertaken over a period of a year or longer, whilst others have been short point-in-time studies which may not reflect the true situation under a variety of environmental and seasonal conditions. This survey was done over a year and included two rounds of sampling to capture seasonal variability in viral levels in Australian oysters. As NoV and HAV were not detected in either sampling round, we are unable to comment on seasonal variability in 2014–2015. The low prevalence of NoV and HAV in Australian oysters was however supported by epidemiological evidence, with no Australian oyster-related viral foodborne outbreaks confirmed during the survey period (OzFoodNet unpublished data). No locally acquired cases of hepatitis A were suspected or confirmed to be due to oyster consumption during the survey period and, although two foodborne outbreaks thought to be associated with oysters were suspected, no pathogens were detected in either the cases or the oysters. Recent reports of NoV illness within the Australian community found it be more common during the winter/spring period (state of Victoria) during 2013 (VIDRL, 2013). However, between January 2013 and June 2014 NoV outbreaks within NSW peaked in spring (October), while in Western Australia an increase in NoV was observed in summer (December to March) (Lim et al., 2016). Prevalence of NoV and HAV in oysters from the UK, USA and Ireland is reported to be higher in the colder winter months than in summer (DePaola et al., 2010; Flannery et al., 2009; Lowther et al., 2012). Seasonal occurrence of shellfish associated NoV outbreaks in Europe has been noted and is thought to be attributed to several factors including a higher prevalence of the disease within the community during winter, increased stability of the viruses in cold water, reduced solar inactivation and selective bioaccumulation by shellfish (Lowther et al., 2012; van Beek et al., 2013). In contrast, a retail survey of NoV and HAV in Korean shellfish identified no seasonal variability in virus prevalence (Seo et al., 2014), while an 18 month survey of Spanish bivalve shellfish (mussels, cockles and clams) found higher viral contamination in the shellfish during the warmer summer months than in the winter (Polo et al., 2015).

Only a few recently published studies have quantified the amount of NoV detected in contaminated shellfish (Lowther et al., 2012; Polo et al., 2015; Schaeffer et al., 2013; Suffredini et al., 2014). Available data indicate that NoV concentration in shellfish linked to human cases of illness vary greatly from <100 to >10,000 RNA copies/g DT, yet there is growing evidence of a dose response for NoV (EFSA, 2012). It has been suggested that NoV monitoring of at-risk oyster harvesting areas, along with the introduction of an upper limit for NoV in oysters could prevent a significant number of outbreaks associated with oyster consumption in Europe (Doré et al., 2010). However, the lack of suitable data to date has hampered the development of a quantitative risk analysis and definition of microbiological criteria for viruses in shellfish. The EU is currently undertaking a baseline survey for NoV in oysters to determine prevalence and inform decision making (EFSA, 2016).

Results of this survey indicate that for the period of the study the food safety risk related to enteric foodborne viruses in Australian shellfish was low. Results of the current survey could be used to support trade, increase market access and potentially improve market value by demonstrating the high quality of Australian oysters and the low risk of foodborne enteric viruses. The results of the survey could also be used to argue against the unilateral need for mandatory virus monitoring as being proposed in the EU (CEFAS, 2013; EFSA, 2012). In countries or regions with a demonstrated low risk of viral contamination such monitoring could be considered unnecessary, excessive and costly.

5. Conclusions

This national survey of Australian oysters destined for market resulted in an estimated prevalence of NoV and HAV of <2%, with no virus positive samples detected and no related foodborne illnesses reported. Although, there will always be a risk of foodborne viral illness associated with oysters when product is eaten raw, especially if grown in water that can be impacted by sewage and environmental run-off; the results of this study suggest that the viral contamination risk was low for the period of the survey. Furthermore, there is no evidence that end-product viral limits are necessary in the Australian context. Any end-product viral limits should be applied on a risk assessment basis.

Acknowledgments

We acknowledge the financial support of the Fisheries Research and Development Corporation (grant number: FRDC 2013/234), Oysters Australia, Tasmanian Oyster Research Council, Tasmanian Shellfish Executive Committee, South Australian Oyster Research Council, South Australian Shellfish Quality Assurance Program and New South Wales Food Authority. We acknowledge the Australian oyster industries and state Shellfish Quality Assurance Programs for their in principle support of the project and provision of time and resources in survey sample collection. We acknowledge the technical support of SARDI staff including Ina Dumitrescu, Linda Friedrich, and Joanne Tomkins.

References

ABARES, 2012. Table 1: Gross Value of Fisheries Production. http://data.daff.gov.au/data/warehouse/9aam/afstad9aamd003/2012/AFS_Production11Yrs_v1.0.0.xls.

Abelson, P., Potter Forbes, M., Hall, G., 2006. The Annual Cost of Foodborne Illness in Australia. Australian Government Department of Health and Ageing.

ASQAAC, 2016. Australian Shellfish Quality Assurance Program, Operations Manual. http://safefish.com.au/technical-program.

Bellou, M., Kokkinos, P., Vantarakis, A., 2013. Shellfish-borne viral outbreaks: a

systematic review. Food Environ. Virol. 5, 13–23.
Benabbes, L., Ollivier, J., Schaeffer, J., Parnaudeau, S., Rhaissi, H., Nourlil, J., Le Guyader, F.S., 2013. Norovirus and other human enteric viruses in Moroccan shellfish. Food Environ. Virol. 5, 35–40.
Brake, F., Ross, T., Holds, G., Kiermeier, A., McLeod, C., 2014. A survey of Australian oysters for the presence of human noroviruses. Food Microbiol. 44, 264–270.
CEFAS, 2011. Investigation into the Prevalence, Distribution and Levels of Norovirus Titre in Oyster Harvesting Areas in the UK. CEFAS Weymouth Laboratory.
CEFAS, 2013. Discussion Paper on Live Bivalve Molluscs (LBM) and Human Enteric Virus Contamination: Options for Improving Risk Management in EU Food Hygiene Package. European Union Reference laboratory for monitoring bacteriological and viral contamination of bivalve molluscs, Weymouth, UK.
Conaty, S., Bird, P., Bell, G., Kraa, E., Grohmann, G., McAnulty, J.M., 2000. Hepatitis A in New South Wales, Australia from consumption of oysters: the first reported outbreak. Epidemiol. Infect. 124, 121–130.
Costantini, V., Loisy, F., Joens, L., Le Guyader, F.S., Saif, L.J., 2006. Human and animal enteric caliciviruses in oysters from different coastal regions of the United States. Appl. Environ. Microbiol. 72, 1800–1809.
Da Silva, A.K., Le Saux, J.C., Parnaudeau, S., Pommepuy, M., Elimelech, M., Le Guyader, F.S., 2007. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Appl. Environ. Microbiol. 73, 7891–7897.
DePaola, A., Jones, J.L., Woods, J., Burkhardt 3rd, W., Calci, K.R., Krantz, J.A., Bowers, J.C., Kasturi, K., Byars, R.H., Jacobs, E., Williams-Hill, D., Nabe, K., 2010. Bacterial and viral pathogens in live oysters: 2007 United States market survey. Appl. Environ. Microbiol. 76, 2754–2768.
Doré, B., Keaveney, S., Flannery, J., Rajko-Nenow, P., 2010. Management of health risks associated with oysters harvested from a norovirus contaminated area, Ireland, February-March 2010. Eurosurveillance 15, 1–4.
EC, 2004. Annex II of Commission Regulation (EC) No 854/2004 on Laying Down Specific Rules for the Organisation of Official Controls on Products of Animal Origin Intended for Human Consumption. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32004R0854&from=en.
EC, 2005. Commission Regulation (EC) No 2073/2005 on Microbiological Criteria for Foodstuffs. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R2073&from=EN.
EFSA, 2012. Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options. EFSA J. 10 (1), 250, 39 pp.
EFSA, 2016. Technical specifications for a European baseline survey of norovirus in oysters. EFSA J. 14 (3), 4414, 62 pp.
FAO/WHO, 2003. Codex Alimentarius International Food Standards. Code of Practice for Fish and Fishery Products. amended 2016, second ed. Food and Agriculture Organization - World Health Organization, Rome.
FAO/WHO, 2008. Viruses in Food: Scientific Advice to Support Risk Management Activities: Meeting Report. Microbiological Risk Assessment Series. No. 13., Microbiological Risks Assessment Series. Food and Agriculture Organization - World Health Organization, Rome.
FAO/WHO, 2012. Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food, Microbiological Risks Assessment Series. Food and Agriculture Organization - World Health Organization, Rome.
FDA, 2015. National Shellfish Sanitation Program (NSSP) Guide for the Control of Molluscan Shellfish, 2015 revision. http://www.fda.gov/Food/GuidanceRegulation/FederalStateFoodPrograms/ucm2006754.htm.
Flannery, J., Keaveney, S., Dore, W., 2009. Use of FRNA bacteriophages to indicate the risk of norovirus contamination in Irish oysters. J. Food Prot. 72, 2358–2362.
Handmer, J., Hillman, M., 2004. Economic and financial recovery from disaster. Aust. J. Emerg. Manag. 19, 44–50.
Hewitt, J., Rivera-Aban, M., Greening, G.E., 2009. Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. J. Appl. Microbiol. 107, 65–71.
ISO/CEN, 2013. Microbiology of Food and Animal Feed - Horizontal Method for Determination of Hepatitis A Virus and Norovirus in Food Using Real-time RT-PCR - Part 1: Method for Quantification ISO/TS 15216-1:2013, Corrected Version 2013-05-01, CEN/TC 275-Food Analysis - Horizontal Methods.
Lim, K.L., Hewitt, J., Sitabkhan, A., Eden, J.S., Lun, J., Levy, A., Merif, J., Smith, D., Rawlinson, W.D., White, P.A., 2016. A multi-site study of norovirus molecular epidemiology in Australia and New Zealand, 2013-2014. PLoS One. http://dx.doi.org/10.1371/journal.pone.0145254.
Lodo, K.L., Veitch, M.G., Green, M.L., 2014. An outbreak of norovirus linked to oysters in Tasmania. Commun. Dis. Intell. Q. Rep. 38, E16–E19.
Loutreul, J., Cazeaux, C., Levert, D., Nicolas, A., Vautier, S., Le Sauvage, A.L., Perelle, S., Morin, T., 2014. Prevalence of human noroviruses in frozen marketed shellfish, red fruits and fresh vegetables. Food Environ. Virol. 6, 157–168.
Lowther, J.A., Gustar, N.E., Powell, A.L., Hartnell, R.E., Lees, D.N., 2012. Two-year systematic study to assess norovirus contamination in oysters from commercial harvesting areas in the United Kingdom. Appl. Environ. Microbiol. 78, 5812–5817.
Maekawa, F., Miura, Y., Kato, A., Takahashi, K.G., Muroga, K., 2007. Norovirus contamination in wild oysters and mussels in Shiogama Bay, northeastern Japan. J. Shellfish Res. 26, 365–370.
McLeod, C., Polo, D., Le Saux, J.C., Le Guyader, F.S., 2017. Depuration and relaying: a review on potential removal of norovirus from oysters. Compr. Rev. Food Sci. Food Saf. 16, 692–706.
Moon, A., Hwang, I.G., Choi, W.S., 2011. Prevalence of noroviruses in oysters in Korea. Food Sci. Biotechnol. 20, 1151–1154.
Muniain-Mujika, I., Girones, R., Tofino-Quesada, G., Calvo, M., Lucena, F., 2002. Depuration dynamics of viruses in shellfish. Int. J. Food Microbiol. 77, 125–133.
OzFoodNet, 2006. The Annual Cost of Foodborne Illness in Australia - 7.2 Industry Costs of Contaminated Oysters in Wallis Lake. http://www.ozfoodnet.gov.au/internet/ozfoodnet/publishing.nsf/Content/annual-cost-foodborne-illness.htm~annual-cost-foodborne-illness-07.htm~annual-cost-foodborne-illness-07.2.htm.
OzFoodNet Reports, OzFoodNet Annual Reports 2001-2010, http://www.health.gov.au/internet/ozfoodnet/publishing.nsf/Content/reports-1.
Pavoni, E., Consoli, M., Suffredini, E., Arcangeli, G., Serracca, L., Battistini, R., Rossini, I., Croci, L., Losio, M.N., 2013. Noroviruses in seafood: a 9-year monitoring in Italy. Foodborne Pathogens Dis. 10, 533–539.
Polo, D., Alvarez, C., Diez, J., Darriba, S., Longa, A., Romalde, J.L., 2014. Viral elimination during commercial depuration of shellfish. Food Control 43, 206–212.
Polo, D., Varela, M.F., Romalde, J.L., 2015. Detection and quantification of hepatitis A virus and norovirus in Spanish authorized shellfish harvesting areas. Int. J. Food Microbiol. 193, 43–50.
Schaeffer, J., Le Saux, J.C., Lora, M., Atmar, R.L., Le Guyader, F.S., 2013. Norovirus contamination on French marketed oysters. Int. J. Food Microbiol. 166, 244–248.
Seo, D.J., Lee, M.H., Son, N.R., Seo, S., Lee, K.B., Wang, X., Choi, C., 2014. Seasonal and regional prevalence of norovirus, hepatitis A virus, hepatitis E virus, and rotavirus in shellfish harvested from South Korea. Food Control 41, 178–184.
Shin, S.B., Oh, E.G., Yu, H., Son, K.T., Lee, H.J., Park, J.Y., Kim, J.H., 2013. Genetic diversity of noroviruses detected in oysters in Jinhae Bay, Korea. Food Sci. Biotechnol. 22, 1453–1460.
Suffredini, E., Lanni, L., Arcangeli, G., Pepe, T., Mazzette, R., Ciccaglioni, G., Croci, L., 2014. Qualitative and quantitative assessment of viral contamination in bivalve molluscs harvested in Italy. Int. J. Food Microbiol. 184, 21–26.
van Beek, J., Kroneman, A., Vennema, H., Koopmans, M., 2013. Noronet Report, April 2013. National Institute for Public Health and the Environment, Bilthoven, The Netherlands. http://www.rivm.nl/en/Documents_and_publications/Common_and_Present/Publications/Centre_for_Infectious_Disease_Control/Noronet_updates/NoroNet_update_april_2013.
VIDRL, 2013. The VIDRL Norovirus Report July 2013. Victorian Infectious Diseases Reference Laboratory. http://www.vidrl.org.au/surveillance/norovirus-surveillance/.