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National survey of foodborne viruses in Australian oysters at production

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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.

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).

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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