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A survey of Australian oysters for the presence of human noroviruses

Felicity Brake [a, b], Tom Ross [a], Geoffrey Holds [b], Andreas Kiermeier [b, 1], Catherine McLeod [b, *]

[a] Tasmanian Institute of Agriculture-School of Agricultural Science, University of Tasmania, Private Bag 51, Hobart 7001, Tasmania, Australia
[b] South Australian Research and Development Institute, G.P.O Box 397, Adelaide, South Australia 5001, Australia

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ABSTRACT

Impending international policies for norovirus in oysters and the lack of Australian data suggested there was a need to undertake a national survey of norovirus in oysters. Two geographically distinct oyster-growing areas from each of three Australian states were sampled on 4 occasions during 2010 and 2011. The sites selected were considered by state shellfish authorities to be the most compromised with respect to the potential for human faecal contamination as identified by shoreline surveys. The oysters were tested for norovirus GI, GII and *Escherichia coli*. Norovirus GII was detected in two of 120 (1.7%) samples and norovirus GI was not detected. One of the norovirus positive samples was cloned and sequenced as GII.3. Five of 120 (4.2%) samples were found to have more than the guidance concentration of 230 *E. coli* per 100 g of shellfish but these samples did not contain detectable concentrations of norovirus. The apparently low prevalence of norovirus in oysters from Australian growing areas supports epidemiological data that suggests norovirus contamination of Australian oysters is rare. The results from this study emphasise the need for future norovirus control measures for shellfish to be commensurate with the risk associated with the growing area.

1. Introduction

Noroviruses are a major cause of both sporadic and epidemic gastroenteritis (Atmar, 2010). The viruses, which constitute a genus of the *Caliciviridae* family, are small, nonenveloped and contain single stranded RNA. Noroviruses are environmentally stable (Lopman et al., 2012 and references therein), and are shed in high numbers by infected individuals (10^5–10^{11} viral copies per gram of faeces). These factors and an estimated high infectivity ($ID_{50} \approx 18$ viral particles) (Teunis et al., 2008; Thebault et al., 2013) contribute to noroviruses being the leading cause of foodborne disease outbreaks (Scallan et al., 2011). It is notable that foodborne outbreaks have the highest illness burden of all norovirus outbreaks (Matthews et al., 2012).

Contaminated shellfish caused 13% of all foodborne disease outbreaks of norovirus due to a single food item in the USA from 2001 to 2008 (Hall et al., 2012). Not only is the impact of a foodborne outbreak a concern, but transmission to the rest of the community is an additional burden. An outbreak of norovirus illness linked to steamed oysters showed that secondary cases were observed in 20% of households and 14% of household members as a result of contact with primary cases (Alfano-Sobsey et al., 2012). The reproduction number, which is the mean number of susceptible persons who become infected by an infectious individual, for norovirus is likely to vary greatly (Moe, 2009) with 2 reported in hospital settings (Sukhrie et al., 2012) and 14.05 observed at a world scout jamboree (Heijne et al., 2012). Contamination with norovirus can occur at any stage of the retail chain although most contamination events occur when untreated human faeces flows into the shellfish growing areas (Le Guyader et al., 2000; Butt et al., 2004; Ueki et al., 2005).

Escherichia coli and faecal coliform concentrations are used as regulatory food safety criteria for oysters and their growing areas. They are also considered a useful microbiological indicator of faecal contamination in oysters and seawater immediately following faecal contamination events (Le Guyader et al., 2006). However, concentrations of *E. coli* and coliforms in oysters and growing waters can be reduced within a few days due to elimination and inactivation under tidal and environmental influences (Burkhardt et al., 2000; Crowther et al., 2001; Bougeard et al., 2011; Campos

* Corresponding author. Seafood Safety Assessment Ltd., Isle of Skye, United Kingdom.
E-mail addresses: Felicity.Brake@sa.gov.au (F. Brake), Tom.Ross@Utas.edu.au (T. Ross), andreas.kiermeier@gmail.com (A. Kiermeier), seafoodsafetyassessment@gmail.com (C. McLeod).
[1] Present address: Statistical Process Improvement Consulting & Training Pty. Ltd., Adelaide, South Australia, Australia.

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et al., 2011), whereas the norovirus genome can be detected in oysters for up to 2 months following contamination (Greening et al., 2002, Nappier et al., 2008). The extended retention of norovirus may be due to strain specific binding to tissue-specific carbohydrate moieties present in oysters (Maalouf et al., 2011). Illnesses related to the consumption of shellfish contaminated with norovirus which met *E. coli* end product standards have been reported (Grohmann et al., 1981; Dore et al., 2010; Le Guyader et al., 2010). Due to the inadequacies of *E. coli* as an indicator for norovirus contamination, it has been proposed that norovirus testing should also be incorporated into shellfish risk management programmes (Anonymous, 2012).

The proposed inclusion of norovirus testing into shellfish risk management programmes has driven the development of standard approaches to sampling and testing. To fulfil this need, in 2004 the European Committee on Standardization (CEN) formed a Technical Advisory Group (TAG) to develop a Standard method for the detection of norovirus in a range of at-risk food commodities including shellfish. The method, ISO/TS 15216 has been recently published (Anonymous, 2013a, Anonymous, 2013b) and is undergoing inter-laboratory validation (Anonymous, 2008, updated May 2011). The availability of the standard method will enhance confidence in norovirus data generated to support risk management decisions.

In Australia the conditions for harvesting oysters from all growing areas are regulated by government authorities in each state following guidance in the Australian Shellfish Quality Assurance Program (ASQAP) (Anonymous, 2009). Regular monitoring of *E. coli* and/or faecal coliforms in oysters and growing waters is undertaken as part of the ASQAP (Anonymous, 2009). However, limited testing of norovirus in Australian oysters has been undertaken.

The aim of this study was to assess the occurrence of norovirus and *E. coli* in *Crassostrea gigas* (Pacific oyster) or *Saccostrea glomerata* (Sydney Rock Oyster) from two of the most compromised oyster-growing areas in each of three Australian States ($n = 6$). The oysters were tested for norovirus GI and GII using the method developed by Greening and Hewitt (2008) which utilizes real-time PCR. The method is closely aligned with the ISO/TS 15216 (Anonymous, 2013a, Anonymous, 2013b) and has been shown to be both accurate and sensitive for detecting norovirus in shellfish (Greening and Hewitt, 2008). The data from this survey provides knowledge of norovirus and *E. coli* prevalence in Australian oysters at the production level and can be used to inform future risk assessment and norovirus risk management strategies.

2. Material and methods

2.1. Shellfish sampling

Six geographically distinct oyster-growing areas, selected from Australia's three largest oyster producing states, New South Wales (NSW), Tasmania (TAS) and South Australia (SA), were chosen for this study. All selected sites were considered by shellfish authorities in their respective states as the most compromised with respect to the potential for human faecal contamination as identified from the shoreline surveys and were classified according to the criteria contained in the ASQAP Operations Manual (Anonymous 2009) (Table 1). This approach was used to maximise the chances of detecting norovirus and *E. coli*, as their occurrence was considered to be unlikely given the lack of human illness outbreaks linked to oysters in Australia.

Table 1
Location and parameters of oyster sampling and sites for the study.

Oyster growing area	Classification as per ASQAP	Species of oysters sampled	Human population within the catchment area	Water temperature °C winter – summer	Flushing rate (tidal cycles)	Sampling dates		Additional information
						Summer Autumn	Winter Spring	
TAS 1	Conditionally approved	Pacific oysters	2050	11–16[a]	10[a]	16/02/10 27/04/10	28/07/10 22/09/10	Situated adjacent to STP outfall
TAS 2	Conditionally approved	Pacific oysters	1,020	7–21[a]	1.4[a]	16/02/10 27/04/10	29/07/10 12/09/10	
SA 1	Closed, inactive	Pacific oysters	200	12–24	4.3[b]	2/02/10 4/05/10	6/08/10 2/11/10	Nursery site for cultivating oyster spat. Water temperature based on local knowledge of the oyster farmer
SA 2	Conditionally approved	Pacific oysters	500	12–24	4.3[c]	2/02/10 4/05/10	6/08/10 11/11/10	Water temperature based on local knowledge of the oyster farmer
NSW 1	Unclassified	Sydney rock oysters	1,200,000	15–25[d]	1[d]	27/01/10 20/04/10	18/08/10 27/10/10	Confirmed outbreak of gastroenteritis in 1978–79. The area is used for ongrowing. Oysters are required to be translocated to approved areas for 60 days prior to harvesting.
NSW 2	Prohibited	Sydney rock oysters	6500	14–27	2–5[e]	27/01/10 20/04/10	13/07/11 6/12/12	Situated adjacent to STP outfall. The water temperature is based on local knowledge of the oyster farmer. The area is used for ongrowing. Oysters are required to be translocated to approved areas for 60 days prior to harvesting.

Data obtained from.
[a] Crawford and Mitchell (1999).
[b] Oceanique Perspectives (1999).
[c] Luick and Middleton (2010).
[d] Anonymous (1995).
[e] Manly Hydraulics Laboratory (2002).