Fact Sheet
Survey of Foodborne Viruses in Australian Oysters

Aims of the project
1. To design a statistically robust survey to evaluate virus occurrence in oyster growing areas in NSW, Qld, SA and Tas
2. To identify the prevalence of human norovirus (NoV) and hepatitis A virus (HAV) associated with Australian oysters at harvest
3. To use the survey results to support trade and market access of Australian oysters

What are the benefits of the project?
Currently there is little information on the baseline level of viruses in Australian oysters, although a pilot project that targeted two high risk areas from each of SA, NSW and Tas indicated a low prevalence (<2%). Similar surveys have been undertaken worldwide, including in the USA, UK, France, China and NZ and have been used to develop or implement regulation.

There is considerable discussion on the international scene for regulation of viruses in bivalves. The European Food Safety Authority has recommended the introduction of an acceptable NoV limit in oysters and the EU Community Reference Laboratory has recommended an absence criterion be applied for HAV in bivalves. Furthermore, an international standard (ISO/TS 15216) was published in 2012 for testing of NoV and HAV in bivalves and the Codex Committee on Food Hygiene released guidelines this year on viruses in foods, with a specific annex on bivalves.

This survey will:
- Provide assurance to overseas markets that we are implementing international best practice by examining the level of risk from enteric viruses in Australia to determine if further management practises are necessary,
- Contribute to the development of market access strategies at the international level,
- Provide an opportunity for pollution source remediation if virus hot spots are identified, hence contributing to reducing cost of impacts.

What will results mean?
The result will provide information on a baseline level of NoV in Australia shellfish growing areas. A low prevalence will support the argument that further regulation (including regular testing) is unnecessary. Higher prevalences will indicate that more work should be conducted to better manage this issue, at least in some areas.

Sample details and time-frame
Sampling will be done of oysters sourced at the production area level and not at retail. Samples are proposed to be taken in each of the main oyster producing states (NSW, SA, Tas and Qld). 300 samples will be taken over a 12 month period in the calendar year of 2014. 150 samples will be taken during summer and autumn and 150 samples during spring and winter to capture both ‘peak’ and ‘off peak’ NoV seasons within the community. Sampling of 150 oysters (in each of the two periods) will ensure that at least one positive NoV result is obtained if >2% of oysters grown in Australia are contaminated with NoV. Conversely, if all results are negative, this will indicate that no more than 2% of oysters produced during the study period were contaminated with NoV.

How will production areas be chosen?
The contribution of each growing area in each state to total oyster production (by volume) will be determined over the previous 5 year period. This proportion of national production will be used to determine the growing areas to be sampled. The production area volumes will inform a randomised sampling program which is weighted for each area by five-year average production volumes. This approach will facilitate representative sampling based on recent production. The sampling program eliminates non-random sampling bias and therefore enhances the scientific robustness of the study. This approach will likely mean that samples are taken from a range of different production area classifications (e.g. approved, conditionally approved, etc.)

What will be done with samples?
Samples will be sent to SARDI for testing. One sample will comprise six individual oysters. Samples can be frozen as this will not affect analysis. Once samples are received they will be stored in a freezer, and batch analysed for NoV and HAV using a molecular method which detects the viral genome. There will be a minimum of one month between sampling and analysis, although this time frame is likely to be greater. Detection is based on an international standard released in 2012 (ISO/TS15216: Microbiology of food and

Prepared by South Australian Research & Development Institute with Oysters Australia and NSW Farmers Association
animal feed - Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR). These methods are able to detect and quantify NoV and HAV in shellfish. The limit of detection of the method is approximately 100 viral genomes per gram of shellfish gut.

**What happens to positive results?**
There are no limits for NoV currently prescribed in the Australian Food Standards Code, or the Australian Shellfish Quality Assurance Operations Manual. Any action on positive results will be determined by the local state regulator. A communication protocol for such events will be developed in consultation with the steering committee, which will include both industry and regulatory representative.

The regulator will need to interpret the results in light of the limits of the test method, the date of sampling, the sanitary survey for the growing area, any other relevant information from the area (recent microbiological results, environmental events, sale history, etc).

**Selection and role of steering committee**
Members of the steering committee will be established through consultation with the oyster industry stakeholders prior to commencement of the project (if approved). It could include a national oyster industry representative, a state oyster industry representative from each participating state (SA, NSW, Tas, Qld), State regulators and Department of Agriculture representatives. The role of the committee will be to provide strategic oversight of the project, assist with communicating results of the study to industry and other stakeholders and provide guidance to project researchers and industry on protocols for handling commercially or market sensitive information arising from the project. The role of the steering committee will not be to drive the science of the research although feedback on project design will be sought.

**State meetings to communicate project**
The key researcher will travel to participating states (Sydney, Hobart and Port Lincoln) at the beginning of the project to meet with regulators and shellfish growers with the aim of communicating the project and establishing the sampling plan. Outcomes of the project will also be communicated to both industry and regulators through presentation at annual state shellfish conferences.
Frequently Asked Questions
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Is there any information on the prevalence of NoV in Australia and the risk to shellfish production?
In Australia it has been estimated that 32% of all reported gastroenteritis is foodborne, accounting for 5.4 million cases, 15,000 hospitalisations and 80 deaths annually. Of these, NoV accounts for approximately a quarter of foodborne gastroenteritis annually. Between 1980 and 2012 there were 368 reported shellfish associated viral gastroenteritis outbreaks reported in the international scientific literature. The most common viral pathogens involved were NoV (84 %) and HAV (13 %) with the most frequent shellfish implicated in outbreaks being oysters (58%). Between 2001-2010 seventeen Australian cases of suspected shellfish related NoV outbreaks were reported in OzFoodNet. In some cases, where NoV was confirmed as the cause of illness, frozen imported oysters were implicated. More recently consumption of oysters from Camden Haven (NSW) caused 36 people to suffer from NoV illness (2012) while over 500 people were reportedly affected by NoV following consumption of contaminated oysters from Tasmania (2013).

Is there a link between faecal coliform levels and norovirus?
There is weak correlation between presence of faecal coliforms (E. coli) and the presence of NoV in waterways. Faecal coliforms will be present in all faecal contamination events; however, NoV will only be present if it is circulating within the human population. Furthermore, faecal coliforms are less stable in the environment and hence relatively short lived following a contamination events, whereas NoV, survives for longer meaning NoV may still be detectable when faecal coliforms are no longer present. NoV has also been shown to be selectively accumulated and retained within the digestive tissues of oysters, persisting long after bacterial indicators of sewage contamination are no longer detectable. Hence, depuration is not effective in eliminating NoV from shellfish.

Is there a risk of growing waters being contaminated with norovirus?
Faecal contamination of shellfish production areas, especially near highly urbanised locations, results in an increased risk of any NoV that is circulating within the community accumulating in filter feeding shellfish and resulting in foodborne outbreaks. Currently NoV detections do not discriminate between viable (infective) or non-viable (non-infective) virus, but its detection is linked to presence of human sewage in the environment: a high risk situation in oyster growing areas.

Is there a difference between viable and non-viable norovirus and what does that mean?
Yes there is a difference between viable and non-viable NoV as only the viable viruses are able to cause gastroenteritis. NoV is excreted at high levels (≤10^{11} virus particles/g faeces) from infected individuals, and it only takes a few virus particles to cause illness. The median infectious dose of NoV is estimated to be as low as 18 virus particles, although the probability of becoming ill in susceptible individuals is dose-dependent. Currently the best practice for detecting NoV and HAV in shellfish is based on an international standard (ISO/TS15216). Although this analysis is the most advanced methodology available, it is likely to underestimate presence of both viable and non-viable virus. Therefore, the risk of not detecting NoV when it is present at very low levels is potentially greater than detecting non-infectious NoV.

What is the time frame between sampling and analysis?
The proposed work will be run as a research project and not as a diagnostic service. In our current experimental plan we estimate a month lag between sampling and analysis, although this will be generally longer due to batching of processed samples prior to analysis. The benefits of this are that the costs of undertaking the project remain reasonable due to economy of scale. We do have the capacity to do diagnostic testing with a week turnaround; however such work is only done when oysters have been implicated in a NoV outbreak.