

Evaluating the effectiveness of depuration in removing norovirus from oysters

Research programme [Marine Microbiology and Biotoxins Research Programme P01](#)

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Background

The 'foodborne viruses' research programme aims to gather data to provide a robust science and evidence base to inform development of a risk management programme on foodborne viruses, with a particular focus on norovirus. Norovirus is the most common cause of infectious intestinal disease in the UK. It is highly infectious, but the illness is generally mild and people usually recover fully within two to three days. Most norovirus infections are thought to spread from person-to-person, although contaminated foods are also thought to account for a proportion of cases. Food can be contaminated with norovirus by infected food handlers or as a result of contamination occurring during primary production, eg shellfish or other fresh produce. The presence of norovirus in foods eaten raw, such as oysters or salad vegetables, can present a particular risk to consumer safety as the virus is not destroyed before consumption by heat processes such as cooking. The European Food Safety Authority (EFSA) has published two Scientific Opinions on foodborne viruses in 2011 and 2012. These contain a number of recommendations on potential control options for shellfish (particularly those eaten raw) and the need to gather data, including further research to establish and optimise the effectiveness of depuration and relaying for Norovirus (NoV) reduction. Our organisation is working with the UK shellfish industry to facilitate the development and implementation of practical controls to manage the risk from norovirus-contaminated oysters.?

Research Approach

Depuration is effective in reducing bacterial contamination, but is known to be less effective in reducing norovirus. The lack of an effective process capable of reliably removing norovirus from oysters is an important knowledge gap and a priority for further research.

Project objectives:

- Carry out a critical review of international literature to evaluate the effectiveness of depuration in removing norovirus from oysters, the mechanism by which norovirus is specifically bound and retained in oysters, and to identify new tools and/or approaches for viral depuration.
- Complete a series of pilot experiments to investigate depuration of norovirus from Pacific oysters using a novel approach to release norovirus from its specific receptor.
- Facilitate an industry engagement strategy to elicit industry input and disseminate the findings of the literature review and preliminary experiments.
- Develop recommendations on future research and/or field trials that could be undertaken

to underpin the commercial application of depuration by oyster growers.

Proposed approach:

Literature review

A review of the published and non-published literature will be carried out to evaluate the effectiveness of depuration in removing norovirus from oysters, examination of the mechanism by which norovirus specifically binds to ligands within the oyster tissues and to identify potential new approaches and tools for depuration. The literature review component of the project will also provide information that will form basis for selection of the most promising compounds (treatments) to trial in the pilot studies. Key considerations regarding which compounds to trial will include the need to utilise those that are non-toxic to humans at the concentrations used to treat oysters, are of sufficiently broad a range to degrade all ligands in oysters that bind noroviruses/other viruses, and do not significantly alter the sensory attributes of the oysters (eg taste, smell etc.)

Pilot depuration experiments

A series of pilot experiments will be carried out to investigate depuration of norovirus in Pacific oysters using a novel approach that aims to release norovirus from its specific receptor/ligand within oyster tissues. Specific compounds will be trialled immediately before depuration that may destroy these receptors is carried out, avoiding the release of norovirus during depuration. These preliminary studies will investigate whether this approach has the potential to reduce norovirus levels significantly. If these initial studies show efficient reductions in norovirus, further work would be needed to evaluate the commercial application of such a technique.

Results

Findings of the literature review concluded a specific relationship between Norovirus (NoV) and oysters (in comparison to other viruses), and reported on the limited success of previous studies that have focused on optimising operational parameters of the depuration process (i.e. temperature, salinity, feeding etc). Some topics for considering future research to support the production of virologically safe oysters were considered:

- A major focus should be placed on improvements in coastal water quality. Further collaboration between UK water companies, Local Authorities and industry should be prioritised in order to develop cohesive and practical strategies to achieve this goal.
- Collaborative research to investigate the infectivity of NoV in oysters during depuration and relaying.
- Improving understanding of the virus-oyster relationship and specifically the ligand- binding interactions.
- Investigations into post-harvest interventions that aim to disrupt the specific binding of NoV to oysters.

Promising initial results to support future in-depth investigations were identified from the laboratory studies. An in vitro method was developed to test some compounds and depuration trials identified two plant proteases, Proteinase K (PK) and papain, that showed promise in reducing NoV in oysters. The depuration trials demonstrated that trypsin may also be effective. While these initial studies are encouraging, the level of reduction achieved to date is not regarded as significant and further research is required to confirm and optimise the effect of these compounds. While enzymatic pre-treatment may hold potential for optimising depuration processes, it is clear that NoV can persist for long periods in oysters and it is considered that the major focus should be placed on improvements in water quality to avoid contamination of shellfish at source. Some specific recommendations for further research were offered:

As the preliminary experiments were only conducted on oysters contaminated with high levels of NoV, further experiments are required on oysters containing lower levels of contamination, to confirm findings. Quantification of lower levels of NoV in oyster tissues would require use of a more precise method such as digital PCR (dPCR).

Further studies that seek to optimise the physiological activity of oysters and the enzymatic activity of PK, papain and trypsin should be conducted, including optimisation of conditions such as seawater temperature, pH, enzyme concentration, and dipping and depuration periods.

Consideration could be given to conducting experiments which involve the immersion of contaminated oyster batches for short periods in warm seawater, at temperatures at which the enzymes will be more efficient (generally $>30^{\circ}\text{C}$).

Further techniques to ensure that added treatments such as enzymes reach the sites of interest inside the oyster could be explored. Microencapsulation has been used for the delivery of vaccines and probiotic bacteria in the aquaculture sector and holds potential for use in the oyster context.

Research Report

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