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Ms. Denice Regnier
Project and Corporate Administrator
ISLAND COASTAL ECONOMIC TRUST

November 27, 2019

Dear Ms. Regnier

INNOVATION SUPPORT – ICET: FINAL REPORT
NOROVIRUS ASSAY DETECTION TOOL AND BIOSURVEILLANCE PROGRAMME

Please accept the following as our final report for the development of a Norovirus (NoV) abbreviated assay and biosurveillance program development through the *ICET Innovative Support* program.

Summary
Through the project support, we developed an accessible and expedient molecular assay service that detects the human pathogen Norovirus (NoV) in oyster tissue and seawater. To develop this capacity, we first had to 'ground truth' this assay by testing it against real-time infected oysters. As well, had to perfect the 'recipe' of reagents and process - optimization - so that we could later use the assay in a broader biosurveillance program to detect NoV in seawater. The final test was to ensure that the modified NoV assay was on par with other internationally-recognized regulatory organizations and laboratories that test for and are involved in public food safety. The implications for human health and safety in addition to supporting the local oyster growing industry are manifest. To accomplish this, we are thankful for the support of ICET and additional support to that provided by our partners in project: BC Ministry of Agriculture, North Island College and the BC Shellfish Growers Association.

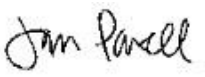
The project was developed in three phases:

1) Optimization of the assay
2) Detection of NoV in water
3) Biosurveillance programme development.

Optimization and proficiency panel testing (comparing to other labs) was completed and the assay validated for detection in oysters. Methods for detection of NoV in seawater were identified and a protocol determined.

The mechanics of NoV detection for use in a biosurveillance programme are ready for implementation. The additional funding for rolling out a biosurveillance pilot were not forthcoming to the partnership. Still, the methods for NoV detection for use in a biosurveillance programme are available when funding opportunities and need arise.

Regards,



Jim Powell, Ph.D.
Chief Executive Officer
BC Centre for Aquatic Health Sciences

Screening test for Norovirus in BC oysters

Background

The British Columbia Center for Disease Control recorded 176 cases of gastrointestinal illness in BC (n=137), Alberta (n=14) and Ontario (n=25) between March and April 2018. Most of the cases were tested positive for Norovirus (NoV) (https://www.canada.ca/en/public-health/services/public-health-notices/2018/outbreak-norovirus-infections-linked-raw-oysters.html). In 2017, 289 gastrointestinal cases were reported in Canada including British Columbia (201), Alberta (40) and Ontario (48) and were linked to oyster consumption between December 2016 and February 2017 (http://www.phac-aspc.gc.ca/phn-asp/2017/outbreak-norovirus-eclosion-eng.php). As a consequence, several shellfish farms and zones have been closed to harvest in British Columbia compromising the local shellfish economy. Last year, Norovirus outbreaks significantly impacted the local oyster industry with an estimated $9.1 million in losses. In addition to the financial crisis, the BC oyster industry reputation was dramatically impacted to the level that US Food and Drug Administration recently released a warning to the consumers to avoid buying oysters provided from Canada.

Norovirus is the leading cause of human gastroenteritis (diarrhea and/or vomiting) worldwide. Shellfish are the main vector for human NoV due to their high water filtration capacity and accumulation of pathogens in their tissues from their surrounding environment. Oysters are the most implicated shellfish organisms involved in human NoV outbreaks because they are consumed in large volumes worldwide.

Objectives

The goal of this project is to provide oyster growers access to a newly-revised NoV detection technology that is accessible, reliable and expedient. The assay fits the purpose of testing oysters collected from the farms for the presence of the virus and provides fast turn-around of results. This allows detection of the virus at the farm level before the virus could become widespread and informs farmers, producers and regulators of any potential threats. Further, the routine monitoring of NoV in oysters could allow a better management of outbreaks in BC and elsewhere, therefore minimizing the dramatic financial and reputation impacts experienced previously. The assay can also help identify the source of the NoV and allow for future mitigation strategies to deal with identified threats to production.

Activities and Deliverables

Phase 1:

Our modified NoV assay was abbreviated from the standard International Standards Organization (ISO) assay and optimized for high performance and sensitivity with multi-level controls. This adaptation required an innovative approach to modify the assay to be more cost-effective, faster and use fewer consumables while remaining accurate, precise and reliable. This adaptation included controls for quality assurance to demonstrate that the presence or absence of NoV detection are accurate.

We tested our assay using Mengo virus as a surrogate – it is a NoV-like virus that acts as a non-contagious control and is non-infectious to humans. While our adapted test has met the required ISO/TC 15216-1:2017 standardized protocol requirements, the remaining step was to prove that the assay could detect NoV in oyster tissue. Without this crucial step of testing real-time NoV, there was no definitive proof that the test is valid.

The Canadian Food Inspection Agency (CFIA) redacted an offer to supply NoV-infected oysters for our work. Instead, we applied to the European Union Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusks in United Kingdom to supply a proficiency panel. The panel is a collection of samples that are contain – or don't contain – NoV contamination and are distributed to laboratories world-wide for comparison testing between labs. The central lab tests the samples for reference values (infected or not infected) and then randomly assigns 'blind' labels to the samples. The panel is then sent to the receiving labs who do not know the uninfected from infected samples. The labs – several dozens world-wide – test the samples and send the results into the European Union Reference Laboratory who then compile and compare the results.

This was the only way we could obtain NoV-infected tissues for validation of our assay. The additional gift of this proficiency panel testing is that we compared our assay to more established laboratories around the globe. The results demonstrated that our abbreviate assay was proficient at testing for the presence of NoV in oyster tissue.

We then tested BC oysters for NoV, which were negative. In this test, we ran the BC oysters with Mengo-spiked material to mimic infection of NoV. This step validated the assay for use in fresh BC oyster products. The extraction and detection methods for the assay were completed and tested and ready for next steps.

Phase 2

To detect NoV in seawater, we enlisted existing methods for extracting DNA/RNA from seawater used for other viruses such as PRv, IHNv, VHSv, etc. The main modification was that the water volumes used are larger for NoV detection. These methods are the basis for the other environmental detection and biosurveillance of marine viruses and are found to be appropriate for use in the NoV biosurveillance programme. Final work to validate this method is dependant on establishing the biosurveillance programme.

Phase 3

Additional funding for developing a biosurveillance programme could not be secured by the project partners. The development team was formed and involved scientists, growers and epidemiologists. However, funding shortfalls external to the project were not secured. The development plans for the programme are on the shelf and ready to further develop and implement when the funding

opportunities and environment are favourable. Noteworthy is that ICET funds were not requested for this part of the study; ICET funds were required to establish the Nov assay for use in the last phase.

Budget

The programme funding was within 6% of target with a minor shortfall covered by BC CAHS. There are no outstanding claims and all invoicing and payments have been received.

Summary

The project has produced a NoV detection tool that is accurate, reliable and available to growers and researchers. As a result of support from ICET and the other project partners, BC CAHS was able to modify an existing NoV assay that was cost prohibitive and time-consuming. The proficiency and accuracy of the assay was demonstrated by comparison with international standards of testing and demonstrated to be effective. The utility of the assay of the assay from biosurveillance and testing programmes awaits future opportunity.