Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation (*Aquavalens*)

Key external stakeholders:
Water supply providers, fresh produce sector, bottled water producers, regulators and policy makers

Practical implications for stakeholders:
- The project demonstrated the utilisation of a concentration technique which facilitates the simultaneous concentration of water samples for detection of bacterial, viral and protozoan pathogens.
- High dissolved organic content may impede the detection of bacterial pathogens by PCR based methodologies.
- In a sampling campaign undertaken in four countries no pathogens were detected in irrigation water samples or in target crops, but generic *E. coli* not considered pathogenic to humans were detected on a number of occasions, indicating potential contamination issues.
- Bacterial contamination arising from irrigation water can survive on crops for a number of weeks following the contamination event.

Main results:
- High dissolved organic content, but not turbidity, influenced the detection of bacterial pathogens by PCR.
- In a sampling campaign undertaken in Ireland, Portugal, Serbia and the United Kingdom there was no detection of the target pathogens *Salmonella*, *E. coli* O157, Norovirus GI or GII or Hepatitis A in any of the analysed samples but generic *E. coli* was detected on a number of occasions.
- Contamination which occurs via irrigation water can persist on the crop for a number of weeks following the contamination event.

Opportunity / Benefit: The scientific knowledge and information generated by this project will help inform the development of management practises and policy to reduce the risks associated with waterborne contamination of food crops.

Collaborating Institutions: National University of Ireland Galway; University of Belgrade, Serbia; Instituto Superior Technico, Portugal; James Hutton Institute, University of Surrey, UK; Genetic PCR Solutions, Spain; other Aquavalens consortium members

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1. Project background

The Aquavalens project (www.aquavalens.org), coordinated by the University of East Anglia, centred around the concept of developing suitable platforms that harness the advances in new molecular techniques to permit the routine detection of waterborne pathogens and improve the provision of hygienically safe water for drinking and food production, that is appropriate for large and small systems throughout Europe. The work was undertaken in a systematic approach, with work packages organised in four different clusters; Cluster 1 focused on the identification of appropriate target gene sequences for detection of viral, bacterial and parasitic pathogens in water and markers that would link pathogens to sources of contamination; Cluster 2 focused on sample preparation detection methods, the integration of these into a technological platform and the development of standardised methods and quality control procedures; Cluster 3 focused on field studies in large and small water systems and food production and Cluster 4 provided an assessment of the risks to European water supplies, the impact of future climate change on risks in drinking water and also demonstrated the value of the developed platforms to European public health and Water Safety Plans.

The Aquavalens developed methodology assessed by Teagasc includes a filtration step and secondary concentration, followed by qPCR for individual pathogens which enabled testing for viral, bacterial and protozoan pathogens from a single filtrate. As part of Cluster 3 Teagasc led the work focused on assessing the utilisation of the methodology in the food industry, and in particular industries which are vulnerable to waterborne pathogen contamination events.

An initial review focused on identifying the pathogens of greatest concern and the crops most commonly associated with foodborne disease outbreaks. This informed the selection of the pathogen panel and target crops for inclusion in the sampling campaign. The pathogen panel included E. coli O157, Salmonella, Cryptosporidium, Norovirus GI and GII and Hepatitis A and irrigation and processing water was assessed from crops including sprouted seeds, salad leaves and soft fruits. Bottled water was also examined. The presence of E. coli was also assessed using the Aquavalens developed methodologies. A sampling plan was prepared to ensure standardisation of sampling across jurisdictions and a sampling campaign was undertaken in Ireland, Portugal, Serbia and the United Kingdom, sampling each grower’s site on four occasions over the growing season. The results were compiled by Teagasc, as well as user feedback regarding method implementation. This work was complemented by an assessment of the impact of turbidity and dissolved organic content (DOC) on method performance and an irrigation water trial undertaken on lettuce, where pathogen surrogates were spiked in the irrigation water on one occasion and survival was monitored in water and on the plants during growth, using both novel and conventional methodologies.

2. Questions addressed by the project:

- What crops and pathogens are most likely to be associated with fresh produce related outbreaks?
- Are the concentration and detection methods developed as part of the Aquavalens project useful for assessing irrigation and processing water or bottled water?
- Does high turbidity or DOC impact on the detection of waterborne pathogens by qPCR?
- Grown in typical Irish conditions, how long can pathogens survive on food crops following a one-time contamination event from irrigation water?

3. The experimental studies:

- The final step in the Aquavalens developed assays is based on qPCR which can be subject to inhibition from various sources. The first study focused on whether high turbidity or high dissolved organic content (DOC) water would impact on the detection of pathogens. Water samples with varying turbidity/DOC were spiked with known levels of E. coli or Salmonella and the detection levels were compared for each sample. The impact of a proprietary inhibition buffer was also assessed.

- As water is a critical component of fruit and vegetable production and processing this sector was selected for analysis as part of Aquavalens. A comprehensive literature review was undertaken to identify the crops and pathogens most commonly linked with fruit and vegetable associated foodborne disease outbreaks, as well as the route of contamination, if known. This informed the selection of a pathogen panel and target crops for inclusion in the sampling campaign. Arising from this a standardised sampling plan was developed for implementation in Ireland, Portugal, Serbia and the United Kingdom. The target pathogens were E. coli O157, Salmonella, Cryptosporidium, Norovirus GI and GII and Hepatitis A and irrigation and processing water was assessed from crops.

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including sprouted seeds, leafy greens and soft fruits. The presence of *E. coli* was also included as an indicator organism.

- Sampling campaigns in each country were tailored to the specific production characteristics and availability/seasonality of each crop’s production. A total of 148 irrigation water samples were collected and analysed by all the partners. Additionally, 61 food samples were collected and analysed. The samples were assessed using the Aquavalens developed methodology and a standard cultural method for comparative purposes.

- Temperature plays an important role in pathogen survival in the environment. However, many irrigation trials studying pathogen survival are undertaken in warmer climates than Ireland and therefore not necessarily representative of what may occur in typical Irish climatic conditions. An irrigation trial was undertaken where lettuce plants were irrigated on one occasion with water spiked with known levels of a soil *E. coli* isolate or a cabbage derived *L. innocua* isolate. These strains were used as surrogates for pathogenic *E. coli* and *Listeria monocytogenes*. The survival of the target bacteria was assessed in lettuce plants and in the stored water samples using culture methods (enrichment and/or direct quantification) and the Aquavalens methodology, with 8 sampling points up to 28 days.

**Main results:**

- High DOC, but not turbidity, influenced the detection of bacterial pathogens by PCR. In such cases the use a proprietary buffer improved pathogen detection. This demonstrates the importance of the use of appropriate process controls to ensure that PCR inhibition does not occur, potentially masking pathogen detection.

- There was no detection of the target pathogens *Salmonella*, *E. coli* O157, Norovirus GI or GII or Hepatitis A in any of the analysed samples. One irrigation water sample from the UK was PCR positive for *Cryptosporidium*, but this was not verified by other methods. *E. coli* is a commonly used indicator of the presence of faecal contamination in water. The inclusion of the *E. coli* species kit in addition to the pathogen tests proved valuable for comparative purposes, as a number of samples from three countries tested positive for this indicator. *E. coli* detection in these samples could be explained by the nature of the water samples collected, and their contact with organic matter (untreated irrigation water, spent irrigation water, product washing water, etc.). Looking specifically at the 93 water samples tested in Ireland all samples were negative for any of the target pathogens. Ten samples tested positive for low levels of generic *E. coli* but these were in samples from pre-treatment water and spent wash water where a higher microbial account would be expected.

- The irrigation trial clearly demonstrated the importance of ensuring high quality irrigation water. Survival of both strains was demonstrated up to 28 days following the contamination event (lettuce plants were harvest-ready at this stage). Direct quantification with culture methods showed a 4-log decrease in the concentration of *E. coli* 14 days after inoculation, and a 3-log decrease in the concentration of *L. innocua* 10 days after inoculation. The quantitative PCR results for both strains seem to correlate well with culture based methodologies, although in general the levels were higher than that obtained by cultural methods. In a parallel study monitoring the bacterial survival in water *E. coli* could not be detected by cultural means after three days, but *L. innocua* could be detected for up to 28 days.

4. **Opportunity/Benefit:**

   The project clearly demonstrated the importance of using high quality irrigation water, to ensure product safety. Results indicate that a single contamination event with either *E. coli* or *L. innocua* is detectible up to 28 days later in an autumn grown lettuce crop. Therefore it is essential that all fresh produce producers maintain high levels of water quality, and where these high levels are not naturally occurring install appropriate water treatment technologies to reduce the risk of product contamination.

5. **Dissemination:**

Microbial Contamination of Fresh Fruits and Vegetables. Bernardino Machado-Moreira, K.G. Richards, F. Abram and C.M. Burgess. IUFoST 2016 World Congress of Food Science and Technology, Dublin, Ireland,

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Application of qPCR for the detection and quantification of *Escherichia coli* O157 in irrigation water


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Application of qPCR for the detection and quantification of *Escherichia coli* O157 in irrigation water


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