Aquavalens Project

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation."

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Deliverable D2.1

Determine maximum acceptance levels for genome copy numbers of viruses in water.

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D 2.1. Determine maximum levels for genome copy numbers of selected target waterborne viruses that represent an acceptable risk for the consumers

Background.

One of the specific objectives addressed in Work Package 2 is to establish threshold maximum acceptance levels for genome copies of health-significant waterborne viruses that cannot be grown in cell cultures so that they represent an acceptable risk for the consumers. For some of our target viruses, notably noroviruses (NoV), it would be of public health interest to establish acceptance threshold of genome copy numbers for viruses detected in water and devoted to consumption.

At the time of writing the Aquavalens application, the European Food safety Authority (EFSA) had tasked an expert panel to study the possibility to specify acceptable genome copy numbers for norovirus in oysters. The EFSA expert panel concluded that it is not possible nowadays to establish such threshold acceptance NoV levels. In their conclusions (Scientific Opinion on Norovirus (NoV) in oysters: methods, limits and control options, EFSA Panel on Biological Hazards – BIOHAZ, EFSA Journal 2012;10(1):2500) the expert panel stated that PCR-based detection methods do exist for NoV in bivalve shellfish and that harmonization and standardization are currently ongoing for NoV in shellfish. Actually, the publication of ISO reference methods for NoV (and hepatitis A virus – HAV) was in April 2013

(ISO/TS 15216-1 and -2:2013). With the appropriate quality assurance measures, including accreditation and proficiency testing, the so called standardised CEN method is considered by the EFSA expert panel suitable for use for detection and quantification of NoV in bivalves within a legislative context. It must be added that several Aquavalens partners actively contributed to the development of the standardized reference methods for the molecular detection of NoV and HAV in the CEN/TC275/WG6/TAG4 Committee. One of the matrices for which the methods were developed is water.

The EFSA Panel also recommended that research needs to be conducted to establish the relationship between detection of NoV in oysters by PCR and human health consequences.

In the absence of an infectivity assay to determine the infectivity of human NoV, several volunteer studies have been conducted. In the most recent and complete of these studies (Teunis, 2008) it was shown that exposure of human volunteers to serial dilutions of the original inoculum of NoV (characterized as Norwalk virus, i.e., genotype 1 within genogoroup I; GI.1) yielded a dose-dependent probability of becoming ill ranging from 0.1 (at a dose of 10^3 NoV genomes) to 0.7 (at a dose of 10^8 virus genomes).

On the question of NoV limits, the EFSA Panel concluded that in spite of the above mentioned estimations of dose-dependent probability of acquiring a NoV gastroenteritis, this probability depends also on the characteristics of the organism, the food or water matrix and the host factors. The relationship between the number of infectious virus particles and the number of virus genome copies detected by quantitative PCR is not a constant, and may vary depending on environmental conditions including time from the initial release from the host.

Other differences in the probability of infection may be related to the particular NoV strain involved. In the Teunis study with human volunteers, a GI strain was employed while most cases are caused by GII strains, notably genotype GII.4. In studies of outbreaks of waterborne origin caused by NoV GII performed at the University of Barcelona, levels of 5.7x 10^2 genome copies per litre were associated with an attack rate of 80%, while levels of 2.9x10^6 genome copies per litre were associated with an attack rate of 40%. This apparent paradox may be due to differential pathogenicity of different NoV strains within GII. While the strain responsible for the former outbreak was typed as GII.6, the strain responsible for the other outbreak could only be characterised as belonging to GII. However, it is more likely, that the differences in the attack rates were a consequence of the infectious status of the detected genome copy numbers.

In conclusion, the number of genome copies detected by quantitative PCR may not relate to infectious NoV particles, and as a consequence the method can only be used to provide an indirect measure of risk, and when considering what is an acceptable level of NoV, it is important to realise that the infectious risk associated with very low level positive as determined by qRT-PCR (< 10^2 genome copies /L) may be overestimated.

NoV are excreted in very high numbers (Atmar et al., 2008). This high excretion of NoV represents a significant health threat since the minimum infectious dose has been reported to be as low as 10–100 genome copies (Teunis et al., 2008). Current wastewater treatments do not ensure complete virus removal (Bosch, 2007), hence viruses become environmental contaminants in numbers high enough to represent a public health threat although low enough to pose serious difficulties for their detection. Water-related diseases are associated with exposure to water environments in many ways which include not only waters used for

drinking (Kukkula et al., 1999) but also those used for agricultural practices such as crop irrigation and food processing (Doyle, 2008; Lynch et al., 2009), eventually resulting in foodborne outbreaks (Li et al., 2012).

References

Atmar, R.L., Opekun, A.R., Gilger, M.A., Estes, M.K., Crawford, S.E., Neill, F.H., Graham, D.Y. 2008. Norwalk virus shedding after experimental human infection. Emerg.Infect.Dis. 14, 1553-1557.

Bosch, A. 2007. Human viruses in water. 1st ed. Elsevier, Amsterdam ; Boston.

Doyle, M.P., Erickson, M.C. 2008. Summer meeting 2007 - the problems with fresh produce: an overview. J Appl Microbiol 105, 317-330.

Kukkula, M., Maunula, L., Silvennoinen, E., von Bonsdorff, C.H. 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. The J Infect Dis 180, 1771-1776.

Li, J., Predmore, A., Divers, E., Lou, F. 2012. New interventions against human norovirus: progress, opportunities, and challenges. Annu Rev Food Sci Technol 3, 331-352.

Lynch, M.F., Tauxe, R.V., Hedberg, C.W. 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect 137, 307-315.

Teunis, P.F., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., Le Pendu, J., Calderon, R.