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Mathematical model for viral depuration kinetics in shellfish: An useful tool to estimate the risk for the consumers

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ABSTRACT

Enteric virus depuration from shellfish is a complex biological process that may be influenced by biological properties of the mollusc and/or virus species. On the basis of previous experimental data, a mathematical model was developed to characterize the kinetics of viral elimination during the depuration process. The experimental data consisted on twenty depuration trials, each with 60 kg of Manila clams (*Venerupis philippinarum*) and mediterranean mussels (*Mytilus galloprovincialis*) previously subjected to bioaccumulation with HAV or MNV-1 (as a surrogate for human norovirus), that were performed in an experimental depuration system during 7 days. It was observed that although viral loads decay along depuration, a residual viral load remains at the end of the process suggesting a decomposition of viral load in a diluted load (susceptible of depuration) and a non-diluted load (unavailable to depurate). The model yielded a general equation, which can predict the viral load at any depuration time knowing the specific filtration rate, dependent on the bivalve species, and specific viral properties. The mathematical model can be combined with quantitative risk assessment calculations to determine the safety of the depurated shellfish, which can be very helpful not only for shellfish producers but also to public health authorities.

1. Introduction

Depuration is a statutory requirement in many countries for marketing of fresh shellfish (Anonymous, 2004). The process exploits the natural shellfish pumping activity to purge their intestinal contents, decreasing the likelihood of infectious agents transmission to consumers. Viral depuration is widely known to be less effective than bacterial depuration and the compliance of shellfish products with current sanitary standards (uniquely based on bacterial indicators) cannot guarantee the viral absence (Loisy et al., 2005; Love et al., 2010; Richards et al., 2010; Schwab et al., 1998; Ueki et al., 2007), a fact evidenced by the periodic outbreaks of Hepatitis A and gastroenteritis following the consumption of depurated shellfish (Chalmers and McMillan, 1995; Heller et al., 1986; Le Guyader et al., 2003, 2006). The severe impact of the viral enteric diseases on human populations has brought awareness by governmental authorities worldwide (EFSA, 2011, 2012; Hall et al., 2014). Recently, a standard method based on real time RT-PCR (RT-qPCR) for Norovirus (NoV) and hepatitis A virus (HAV) quantification in foodstuffs (including shellfish) has been developed, which could be incorporated into EU legislation as a reference method (ISO/TS 15216).

From a virological point of view, shellfish depuration is a complex biological process that may be influenced by biological properties of the mollusc and/or viral species. The efficacy of viral depuration, the time required for a significant reduction of the viral load and the specific behavior of different viruses in different bivalve species are critical issues for the development of new and improved shellfish sanitary controls, purification processes, and the enactment of viral legislation for these products.

Predictive models are increasingly accepted as an integral interdisciplinary knowledge in microbiology. They can reduce the dependence on culture-based techniques, which are limited in the case of unculturable or hardly culturable viruses like NoV and HAV, providing a rapid availability of results required in the framework of food security. From this point of view, such models emerge as useful tools for dealing with the challenge of sanitary viral risk associated with the consumption of shellfish.

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Previous experimental results obtained with different virus and shellfish species showed a similar pattern of depuration (Polo et al., 2014a,b,c). Data showed that viral loads at the initial stage of depuration quickly decay until reaching some threshold of viral contamination that depuration cannot remove, suggesting a decomposition of viral load in a diluted viral load (susceptible of being depurated by physical mechanisms) and a non-diluted viral load (sequestered in non-digestive-tract tissues and thus unavailable to remove by physical depuration).

It was questioned if an undergoing simple law dictating such behavior exists, taking as assumptions how and where virus accumulation occurs and how viral load is removed. A simple model was derived from the following hypothesis: (I) some amount of clean flux of water (proportional to the shellfish filtration rate) enters the digestive tissues (II) the diluted viral load present there randomly diffuses to reach a new isotropic concentration of equilibrium (III) same amount of contaminated flux is spelled and (IV) there could be some non-diluted residual viral load. In these hypothesis a linear differential equation relating viral load variation with time, filtration rate of the shellfish and residual viral load was deduced. The solution to this equation fitted the behavior of the previous experimental data with a high degree of accuracy.

2. Rationale

2.1. Theory model

We propose the simplest model, in which some part of the viral load is bound to the gastrointestinal cells of the digestive tissue by specific viral ligands or receptors or enclosed in the non-conductive compartments outside the conductive lumen torrent (and thus unable to be removed by simple drift) (Le Guyader et al., 2006; McLeod et al., 2009; Tian et al., 2007; Provost et al., 2011) while the rest remains diluted in the lumen of the conductive tissues. Thereby, we decompose virus count in two parts:

$$n = n_d + n_r$$

the subscripts meaning diluted and residual. After the bioaccumulation process the residual viral load goes to a constant value. If environment changes, however, only the diluted part could vary with time.

$$n(t) = n_d(t) + n_r$$

Digestive conductive tissue was idealized as a volume v_d resulting of the sum of all its conductive cavities and a volume v_r representing all the other spaces where the residual viral load could be permanently attached or immobilized. When depuration starts, in an infinitesimal time δt digestive tissue absorbs a δv volume of clean water, meaning that virus concentration in the v_d volume of the digestive tissue instantly decreases to $(n_d(t)/(v_d + \delta v))$. At the same time a similar amount of contaminated liquid is expelled out which contains $(\delta v\, n_d(t)/(v_d + \delta v))$ virus. Thereby, in a small δt the variation in the virus count at the digestive tissue is

$$\delta n(t) \equiv n(t + \delta t) - n(t) = -\frac{n_d(t)}{v_d + \delta v}\delta v$$

Dividing both sides by δt and taking the $\delta t \to 0$ limit when $\delta v \ll v_d$ holds, then

$$\frac{\mathrm{d}n_d(t)}{\mathrm{d}t} = -\frac{n_d(t)}{v_d}\frac{\mathrm{d}v}{\mathrm{d}t}$$

Interested in the total viral load take it into account that, as n_r is constant in time then

$$\frac{\mathrm{d}n(t)}{\mathrm{d}t} = -\frac{n(t) - n_r}{v_d}\frac{\mathrm{d}v}{\mathrm{d}t}$$

The rate of clean water entering the digestive tissue per unit of time, $\mathrm{d}v/\mathrm{d}t$, was assumed to be proportional to the filtration rate (f) of the individual and denoted by α in the fraction bellow:

$$\frac{\mathrm{d}n(t)}{\mathrm{d}t} = -(n(t) - n_r)\frac{\alpha f}{v_d}$$

The parameter $\lambda = \alpha f/v_d$ is denoted as the decay rate of viral load. By solving the above first order linear differential equation, the instant viral load at time t is found

$$n(t) = n_r + (n_0 - n_r)e^{-\lambda t}$$

It is convenient to take the equation in its concentration form instead of absolute viral counts to avoid dependence on initial absolute contamination, by dividing both sides by n_0

$$c(t) = c_r + (1 - c_r)e^{-\lambda t} \quad (1)$$

where $c(t)$ is the total viral load at instant t, value 1 meaning initial concentration, c_r is the constant residual load, λ the decay rate. A particular case consists in the absence of residual load, making $c_r = 0$ in the last equation. Meaning of all used symbols can be found in Table 1.

In this proposed model two separated behaviors were found (Fig. 1). The first one is related to the residual viral load, which can be null or positive, and its value depends, in principle, on the particular ability of a specific viral specie or strain to remain attached to molecular receptors present on gastrointestinal cells of the digestive tissue, penetrate to the non-conductive parts of the digestive tissue like connective tissue or hemocytes or to the particular tolerance or capacity of a certain shellfish species to bioaccumulate certain viral species (e.g. amount and/or distribution of viral ligands or receptors, hemocytes, etc) (Le Guyader et al., 2012; Maalouf et al., 2011; Nappier et al., 2008; Provost et al., 2011; Polo et al., 2014c). The second demeanour concerns only to the ability of the host to drift the dilute content of the viral load, which depends only on kinematic factors like the fraction of filtration which goes through the digestive gland, the filtration rate of the species and the size or the structure of the digestive tissues.

Table 1
Index of symbols and definitions used.

Symbol	Definition
$n(t)$	Total viral load
$c(t)$	Relative viral load
$n_d(t)$	Diluted viral load
$c_d(t)$	Relative diluted viral load
n_r	Residual viral load
c_r	Relative residual viral load
n_0	Total initial viral load
f	Filtration rate
v_d	Volume of the conductive cavities of the DT[a]
α	Ratio of filtration rate entering the DT
λ	Decay rate
I	Infectious factor
c	Cooking factor
n_i	Infectious dose

[a] Digestive tissue.