National Institute for Public Health
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Colophon

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This investigation has been performed by order and for the account of Food- and Consumer Product Safety Authority, within the framework of V/330371/01/VV: viruses in food.

Abstract

Quantitative risk profile for viruses in food

Viruses, similar to bacteria, can pose a risk to human health when present in food, but comparatively little is known about them in this context. In a study aimed at the health risks posed by viruses in food products, the RIVM has inventoried both current knowledge and pertinent information that is lacking. The inventory, which is presented in this report as a so-called risk profile, focuses on three viruses that can be transmitted to humans through food consumption. These are the hepatitis A viruses in shellfish, noroviruses in fresh fruits and vegetables and hepatitis E viruses in pork. The study was commissioned by the Dutch Food and Consumer Product Safety Authority.

General findings
The general finding is that to date it has been difficult to obtain a reliable estimation of the number of viruses in food products. This is partly due to large differences in the methodologies currently used to detect viruses in food products. However, accurate information on the number of viruses in a food product is crucial to a reliable estimate of the health risk. The probability that any one person becomes infected increases with an increasing number of contaminated products, or with an increasing number of viruses per product. The shortcomings of the methods currently used to make such estimations are identified in this report, and recommendations are made for improvements that will enable a more realistic determination.

Factors that increase the likelihood of food becoming contaminated with viruses during production or processing were also studied. For raw or fragile products, such as oysters, or fresh fruits and vegetables, viruses are not inactivated by heating because the foods are not cooked before consumption.

Specific findings on the studied viruses
With respect to fresh produce, it is important to estimate how many noroviruses come into direct contact with the fruits and vegetables through the irrigation system. Another possible but important source that needs to be characterized is the transfer of viruses from hands or tools to the food product during harvesting and/or processing. For hepatitis E virus, it is important to know how many pigs are infected at the time of slaughter as this could result in contaminated pork products. If the hepatitis E virus infection occurs months before slaughter, the pigs would likely have recovered by the time of slaughter and the products would not represent a health risk to the consumer. It is also important to determine the number of hepatitis E viruses per product. In terms of shellfish, it is relevant to know how many viruses are present in the surface waters in which they are cultured and the extent to which they remain in the shellfish up to the moment of consumption.

Keywords:
quantitative risk assessment, norovirus, hepatitis A virus, hepatitis E virus, fresh produce, shellfish

Rapport in het kort

Kwantitatief risicoprofiel voor virussen in voedsel

Net als bacteriën kunnen virussen in voedsel risico's vormen voor de volksgezondheid. Over virussen is echter minder bekend. Het RIVM heeft daarom in kaart gebracht welke kennis beschikbaar is of juist ontbreekt om de volksgezondheidsrisico's te kunnen schatten (risicoprofiel). Hiervoor zijn drie virussen uitgelicht die via voedsel naar mensen kunnen worden overgedragen: hepatitis A-virussen in schelpdieren, norovirussen op verse groenten en fruit, en hepatitis E-virussen in varkensvlees. De inventarisatie is in opdracht van de Nederlandse Voedsel- en Warenautoriteit gemaakt.

Algemene bevindingen
In het algemeen blijkt dat het tot nu toe lastig is om het aantal virussen op producten op een betrouwbare manier te kunnen schatten. Dit komt gedeeltelijk omdat de methoden om de virussen aan te tonen sterk verschillen. Om de gezondheidsrisico's te kunnen inschatten is kennis over het aantal virussen juist nodig. De kans dat iemand ziek wordt is namelijk groter naarmate het aantal producten dat besmet is groter is, of wanneer het aantal virussen per product hoger is. De tekortkomingen van de methoden worden in dit rapport aangegeven en enkele aanbevelingen worden gedaan om de berekeningen van het aantal virussen realistischer te maken.

Verder is geïnventariseerd welke factoren de kans vergroten dat voedsel besmet raakt tijdens de productie of de verwerking ervan. Bij rauwe of kwetsbare producten, zoals oesters, of verse groenten en fruit, is het immers niet mogelijk om de virussen eenvoudig onschadelijk te maken door voedsel te koken.

Bevindingen onderzochte virussen
Specifieker is het bij het norovirus belangrijk te achterhalen hoeveel virussen op groente en fruit terechtkomen via het irrigatiewater. Een andere mogelijke bron is via de handen of gereedschap tijdens de oogst en verwerking. Voor het hepatitis E-virus is het van belang te weten hoeveel varkens tijdens de slachtfase de infectie doormaken en zo besmette producten leveren. Als zij de hepatitis E-infectie eerder doormaken, is de besmetting voorbij en vormt dit geen risico meer voor de consument. Ook is inzicht nodig in de aantallen hepatitis E-virussen per product. Wat de schelpdieren betreft, is het relevant om te weten hoeveel virussen in het oppervlaktewater zitten waarin ze worden gekweekt, en in welke mate deze virussen in de schelpdieren achterblijven.

Trefwoorden:
kwantitatieve risicoschatting, norovirus, hepatitis A-virus, hepatitis E-virus, verse groenten en fruit, schelpdieren

Contents

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Summary

This report describes the results of a literature review that was conducted to collect quantitative data on hepatitis A virus is oysters, hepatitis E virus in pork and norovirus in fresh produce. Many studies on these virus-food combinations have been published to date. The majority of those studies focused on the food product prior to or at consumption, either in response to an outbreak or as a cross-sectional survey. A minority of those studies, however, provides data that can be used to conduct a quantitative risk assessment. For instance, studies report the number of positive samples among all examined, but do not report observed quantities of viruses in samples. If viruses were quantified, then generally the reported absolute quantifications of viruses in samples ignored the imperfection in recovery of viruses from samples and any inhibition during amplification with PCR. In addition, the multifold in detection methods, with differing sensitivities and specificities, make it difficult to compare the results between studies. In this situation, quantitative microbiological risk assessment can be a useful tool to structure available knowledge about the food production chain and use data from several points along this chain to estimate public health risks. Conceptual models were therefore generated for the three food production chains by hypothesizing the most important contamination points. Subsequently, literature was reviewed for each of the hypothesized contamination points and described quantitatively in this report. By using a quantitative microbiological risk assessment framework, the number, and spread therein, of viruses at several points in the three food production chains can be estimated. These estimates can subsequently be combined with food consumption data and representative dose-response models to assess the public health burden posed by the three virus-food combinations.

1 Introduction

Viruses are now well-recognised worldwide causative agents of foodborne outbreaks. In Europe increasing numbers of virus-associated foodborne outbreaks are reported with growing concern from EFSA (EFSA, 2011a). For those outbreaks that were verified, noroviruses (NoV) were the most frequent cause, followed by hepatitis A viruses (HAV). Shellfish, molluscs and products thereof were the most frequently implicated food items.

Contamination of food with foodborne viruses may occur through poor hygienic practices, either by food handlers or during food production (Mead et al., 1999). Both humans and animals may serve as reservoirs for foodborne viruses, leading to contaminated foods directly (e.g., contact transmission, intrinsic contamination with zoonotic pathogens from infected animals) or indirectly through the environment. The environment only serves as a passive vehicle for the viruses, because no viral replication takes place outside warm-blooded hosts.

The levels of evidence for viral outbreaks associated with food consumption differ greatly. There may be solely epidemiological indications for a certain food item to be involved in viral disease cases. Alternatively, a specific virus type or even the same virus variant may be detected in both patient and food. However, this level of evidence has only been obtained in some cases (Craun and Calderon, 2001; De Roda Husman et al., 2007; Tillett et al., 1998).

Due to a range of different problems, attribution of a specific virus to foodborne outbreaks in epidemiological studies is hampered. Therefore, underreporting is most likely in order. Where such epidemiological outbreak investigation and molecular tracing studies fail, quantitative viral risk assessment could aid policy makers in informed risk management decisions.

Quantitative viral risk assessment or QVRA yields a risk estimate from the numbers of infectious, human pathogenic viruses in or on a food commodity (Havelaar and Rutjes, 2008). The classical quantitative microbial risk assessment framework entails hazard identification, hazard characterisation, exposure assessment and actual risk characterisation (ILSI, 1996). The product of the first part, hazard identification, is generally called a risk profile.

Previously, some risk profiles were conducted for foodborne viruses, such as a risk profile on Norwalk-like virus in molluscs (raw) in New Zealand (Greening et al., 2003), on foodborne norovirus infections (HPA, 2004) and for hepatitis E viruses (HEV) (Bouwknegt et al., 2009a). These risk profiles largely include qualitative data to provide evidence that specific virus – food commodity combinations may entail a public health risk. Full quantitative viral risk assessments most likely require much greater data sets and data sets that are currently missing. This was also concluded at an international meeting of experts: undertaking a full quantitative risk assessment for FBV may be premature due to data limitations (FAO/WHO, 2008). By composing a so-called quantitative virus risk profile, the available quantitative data along the virus–food commodity production chain can be inventoried. These data can be used to estimate parameters for parts of the quantitative risk assessment model. Furthermore, data gaps will thus be identified.

Here, the three possible transmission routes for foodborne viruses were selected as a subject for a quantitative risk profile. These three were chosen for their different mechanism of transmission: 1) shellfish that accumulate the virus;

2) pork that may be intrinsically infected with virus; 3) fresh produce that may be contaminated due to unsanitary conditions by food handlers or environmentally due to for instance contaminated irrigation water. The most representative viruses for these routes are HAV in shellfish, HEV in pork and NoV on fresh produce.

2 Hepatitis A virus in shellfish

2.1 Introduction

Hepatitis A virus (HAV), a picornavirus, is a common cause of hepatitis worldwide. Hepatitis A is endemic in developing countries, and most residents are exposed in childhood. In contrast, the adult population in developed countries, such as the Netherlands, demonstrates falling rates of exposure with improvements in hygiene and sanitation. The export of food that cannot be sterilized, such as shellfish, from areas of high to areas with low rates of infection, is a potentially important source of infection.

Hepatitis A is a pathogenic virus for humans and susceptibility to the virus is currently reported to be exclusive for humans and several monkey species (Hollinger and Emerson, 2001). The virus can cause serious illness with symptoms indistinguishable from other viral hepatic diseases. The transmission route for HAV is faecal-oral, which is different from most hepatitis viruses (hepatitis E virus excluded), that are transmitted via blood. After ingestion and uptake from the gastrointestinal tract, the HAV replicates in the liver and is excreted into the bile. Cellular immune responses to the virus lead to destruction of infected hepatocytes with consequent development of symptoms and signs of disease. The disease is often asymptomatic, but there may be a range of clinical manifestations from mild, anicteric infection to fulminant hepatic failure. Management of the acute illness is supportive, and complete recovery without sequelae is the usual outcome. Vaccines are available.

HAV has been linked to the consumption of contaminated foods through outbreak studies (Petrignani et al., 2010a; Petrignani et al., 2010b; Sanchez et al., 2002; Shieh et al., 2007). In Europe, shellfish are among the most frequently implicated food items and HAV is among the most frequent causative agent for virus outbreaks related to consumption of shellfish. Shellfish may become contaminated with viruses, such as HAV, through accumulation during the filter-feeding process in contaminated waters. Subsequent processes such as depuration and relaying do not influence the HAV numbers in shellfish significantly which is also true for some of the other post-harvest treatment processes such as washing. Exposure to the resultant HAV by consumption of the shellfish may lead to infection and disease.

Post-harvest risk management options for shellfish contaminated with human pathogenic viruses, such as HAV, are limited to some forms of cooking, however, often shellfish are consumed raw. To be able to estimate public health risks from consumption of contaminated shellfish by QMRA, quantitative data on virus concentrations in shellfish need to be collected. Therefore, quantitative data on HAV in the different phases of the shellfish production chain from HAV in marine waters to HAV in shellfish on the market were gathered for risk assessments with the purpose of informing risk management.

2.2 Components of the conceptual risk assessment model

The only source of hepatitis A virus in shellfish is surface water used for culturing shellfish that is contaminated with human faecal material. This faecal material may be discharged into surface water through various routes. During heavy rainfall, wastewater treatment plants may overflow discharging untreated

wastewater. Wastewater treatment plants may discharge treated wastewater containing HAV due to the employment of treatment processes with a limited capacity to reduce viral load. Alternatively, recreation near or in these shellfish culturing waters may lead to direct contamination by bathers or sewage disposals from boats.

Subsequently, the surface water is filtered by shellfish for feeding at a certain rate, and each filtered HAV particle has a probability of being retained by the shellfish. Each particle also has a certain probability per unit time of being washed from the oyster again, a process referred to as depuration.

An important aspect for QMRA to consider is the distinction between infectious and inactivated HAV, because infectivity is the determinant of human infection given ingestion. HAV can be present in inactivated form as a certain fraction in surface water, or can become inactivated at a certain rate after being retained in shellfish. Distinction between the inactivated and the infectious form of HAV can be made by different detection techniques for HAV in water and shellfish, PCR for detection of both and cell culture for detection of infectious HAV. The total number of HAV per oyster at the moment of consumption is the expected retained number of HAV corrected for the inactivation.

The dose ingested by a human per consumption event is then the sum of the infectious HAV per shellfish and the amount of shellfish consumed. The availability of quantitative data for describing each of these components was investigated, and the suitability of these data for undertaking risk assessment was evaluated.

Since data on HAV in shellfish were limited, other important gastroenteritis viruses such as NoV were also included in the study.

2.3 Quantification of components

2.3.1 HAV concentration in surface water

HAV is excreted in large numbers by infected individuals, and occurrence of HAV in shellfish culturing waters is due to human faecal contamination. The primary site of replication for HAV is the hepatocyte, and HAV may reach the intestinal tract through the bile in substantial particle concentrations (Hollinger and Emerson, 2001). HAV particle concentrations have been estimated up to 10^8 g^{-1} faeces. Faeces end up in sewage via toilets and sewerage pipe systems. Subsequently, human sewage is discharged either directly or indirectly to shellfish culturing waters. The concentration of HAV in shellfish culturing waters depends upon:

- the incidence of infection in the contributing population;
- the mechanism of faecal collection, treatment and discharge. Viral pollution of coastal waters is higher for communities with a high density of septic tanks (Griffin et al., 1999a; Lipp et al., 2001);
- distance from the sewage release;
- environmental conditions such as temperature, sunlight and flushing/dilution rate.

Concentration of HAV in sewage depends on the prevalence of infection in the contributing population, which varies considerably between communities, and countries. In developing countries, HAV infection is endemic and most individuals are infected in early childhood. In the developed world, however, HAV infection is less common. Interpretation of environmental monitoring data

must take into consideration the likely prevalence rate of HAV amongst sewage contributors to the marine waters.
Prevalence rates of HAV in marine waters have been reported in the literature (Table 1). One study reported a concentration of HAV genome copies per L water of 75 – 730 (Rose et al., 2006), but concentrations of HAV in marine waters would be expected to be subject to wide fluctuations.

2.3.2 Filtration rate of shellfish

Pumping rate (PR) is the volume of water flowing out the exhalent siphon of the shellfish per unit time. Direct measurement of pumping rate is difficult, because it is challenging to measure water flow from the exhalent siphon without affecting flow rates. Direct methods have however been applied including:

- physical separation of exhalant water by means of a rubber apron, and the change in volume in the exhalant water chamber is measured. When using this approach, induced hydrostatic pressure effects can influence the filtration rate (Famme et al., 1986; Jørgensen et al., 1986);
- less direct, but still focused on water flow rates, several methods aimed at quantifying velocity profiles have been applied including *video observation* of particles, *thermistor* probes and *impeller*s.

Indirect methods, based on the disappearance of particles in the water, either in a static or a flow-through system have also been applied. These methods aim to quantify the clearance rate (CR) (the volume of water cleared of particles per unit time) and when the capture efficiency of the shellfish is assumed to be 100%, then the CR is equal to the PR. These methods are the most commonly used methods for quantifying filtration rate and include:

- *flow-through chamber* method where algal concentration is measured before and after passage through the chamber;
- the *suction* method where algal concentration in inhaled and exhaled water is compared;
- the *clearance method* where the change in concentration over time within a single chamber is monitored; and
- the *photo aquarium* and the *steady state* methods where the algal dosing rate required to maintain a constant concentration within a fully-mixed chamber is monitored.

Riisgård (2001) reviewed the studies that had been undertaken at that time with particular attention on methodological approaches for quantifying filtration rate and uncertainties, and reported data that were based on reliable methodological approaches applied under optimal laboratory conditions (Table 2). For a full description of methodology, the reader is referred to that review. These filtration rates were developed in the laboratory under ideal conditions, and therefore are considered maximum rates. Actual rates of shellfish filtration in the environment are likely to be lower due to the influence of local fluctuating, non-ideal, conditions. Filtration and clearance rates of shellfish are highly variable between species, populations and for individual organisms over time depending on size, reproductive state, temperature, salinity, food density and food quality. Overall, pumping rate increases with increasing gill area (Meyhöfer, 1985; Riisgård, 1988).

Depending on the type of shellfish, a regression model from the column 'Regression' in Table 2 can be used to estimate the average maximum filtration rate (L water per hour) based on dry mass weight (in g) or shell length (in mm).

Table 1 Reported prevalence of HAV in marine waters

Location	Method	N	Positive	Reference
Estuaries in Mexico (Huizache Caimanero Lagunary Complex)	RT-PCR	40	70%	(Hernandez-Morga et al., 2009)
Venice Canals	Real time RT-PCT	9	8%	(Rose et al., 2006)
Canals and near-shore waters of the Florida Keys	RT-PCR	19	63%	(Griffin et al., 1999b)
Near-shore waters of Barcelona, Spain	RT-PCR	9	33%	(Pina et al., 1998)

Table 2 Filtration rate (F, L per hour) data reported in the literature for a range of shellfish (modified from (Riisgård, 2001) with reference to original papers)

Shellfish	Method	Regression*	Shellfish size*	L hour^{-1}	Reference
Cardium echinatum	Suction	$F=4.22W^{0.62}$	0.08 – 2.13	0.9-6.7	(Møhlenburg and Riisgård, 1979)
Cardium edule	Suction	$F=11.60W^{0.70}$	0.028 – 0.173	0.95–3.4	(Møhlenburg and Riisgård, 1979)
Mytilus edulis	Suction	$F=7.45W^{0.66}$	0.011 – 1.361	0.4–9.1	(Møhlenburg and Riisgård, 1979)
	Suction	$F=0.0012L^{2.14}$	~17 – 70	0.5-10.7	(Kiørboe and Møhlenberg, 1981)
	Photo-aquarium	$F=7.37W^{0.72}$	0.132 (avg)	1.7	(Riisgård and Møhlenburg, 1979)
Modiolus modiolus	Suction	$F=6.00W^{0.75}$	0.058 – 1.555	0.7–8.4	(Møhlenburg and Riisgård, 1979)
Arctica islandicaus	Suction	$F=5.55W^{0.62}$	0.011 – 1.310	0.3–6.6	(Møhlenburg and Riisgård, 1979)
Choromytilus meridionalis	Clearance	$F=5.37W^{0.60}$ $F=0.006L^{1.58}$	W= 0.02-3.2 L =15-110	0.3–6.7	(Griffiths, 1980)
Perna Perna	Clearance	$F=8.85W^{0.66}$ $F=0.0027L^{1.86}$	W=0.0031–3.4 L=~10 to 120	~0.2–20	(Berry and Schleyer, 1983)
Crassostrea virginica	Clearance	$F=6.79W^{0.73}$	0.063 – 0.994	0.9–6.8	(Riisgård, 1988)
Geukensia demissa	Clearance	$F=6.15W^{0.83}$	0.009-1.039	0.12–6.4	(Riisgård, 1988)
Mercenaria mercenaria	Replac-ement	$F=2.5W^{0.78}$	0.33–4.81	1.05–8.51	(Coughlan and Ad, 1964)
Clinocardium nuttalii	Thermistor	$F=3.1W^{0.80}$	2.47–4.46	6.4–10.3	(Meyhöfer, 1985)
Mytilus californianus	Thermistor	$F=7.9W^{0.72}$	0.98–8.662	7.79–37.4	(Meyhöfer, 1985)
Chlamys hastate	Thermistor	$F=8.7W^{0.94}$	8.77–13.27	14.7–18.5	(Meyhöfer, 1985)

* either mass dry weight (g; *W*) or shell length (mm; *L*)

2.3.3 Retention rate of HAV in shellfish

Table 3 includes a summary of the data identified from studies published in the literature on the bioaccumulation of viruses in shellfish. While a great deal of work has been undertaken on the potential for shellfish to bioaccumulate viruses, no studies were identified that related the accumulation of viruses to the shellfish pumping rate as proposed for the hazard pathway. All studies on bioaccumulation rate related the concentration of seeded virus in the water to the concentration or prevalence of viruses identified in the oyster tissue (Table 3), without explicit quantitative consideration of virus retention efficiency of the shellfish.

The studies show that uptake of viruses occurs quickly with maximum uptake occurring within the first 24 hours (Girolamo et al., 1975); however, rates vary between virus types. Feeding activity is essential for virus accumulation and for

virus clearance (Enriquez et al., 1992). The primary site for accumulation is the digestive tissue (Girolamo et al., 1975), however results of poliovirus accumulation reported by (Girolamo et al., 1975) suggested a diffusion of virus from the digestive region into the body of the oyster.

A study from Enriquez et al. (Enriquez et al., 1992) specifically present detailed data on the retention rate for HAV in mussels. These data were used to calculate the number of HAV per mussel and subsequently the fraction of the total HAV count in the water that is retained by a single mussel per hour of filtration. These estimations assume a constant filtration rate in time. The mean proportion retained was estimated at 0.03 (95% uncertainty interval: 0.01 - 0.20). The latter uncertainty was assessed by employing Markov Chain Monte Carlo (MCMC) simulation using the likelihood function in the so-called Metropolis-Hastings algorithm (Gilks et al., 1996). This uncertainty distribution can be approximated by a lognormal distribution with μ=-3.244 and σ=0.775, although for a more accurate representation of the uncertainty the MCMC posterior should be used (to be obtained as text file from the corresponding author). The most likely distribution that describes the variation in the mean proportion of HAV retained was estimated to be the Beta distribution with α=0.289 and β=8.82.

Important limitations of the available data for this purpose however include:

- no studies accounted for or explicitly reported the recovery efficiency of their detection methods in water or viruses;
- often the total mass of shellfish tissue was not reported, only an inferred concentration of viruses per gram, therefore limiting a particle balance approach.

Removal of viruses from the water column during experimental conditions has been shown to also occur due to passive adsorption to the oyster tissue or shell, and the accumulation of viruses as a result of active filtration only represented approximately one half of the total removed from suspension (Bedford et al., 1978).

2.3.4 Depuration rate of HAV in shellfish

Depuration is the process of placing shellfish in clean water for several days to allow them to purge contaminants. Depuration is effective for the removal of bacteria, however studies show that it is less effective for viruses, and depurated shellfish have been associated with outbreaks of viral illness nevertheless (Grohmann et al., 1981; Le Guyader et al., 2008). Virus are suggested to be removed more efficiently from actively feeding shellfish than from those that are not (Enriquez et al., 1992).

Infectious HAV is removed by 1 Log_{10} unit after seven days of depuration from *Mytilus chilensis*, whereas poliovirus was reduced by >5 Log_{10} over the same duration (Enriquez et al., 1992). Love et al. (Love et al., 2010) bioaccumulated HAV in *C. virginica* and estimated the daily log_{10} reduction of culturable HAV at zero at 12^{o}C and 18^{o}C, and at 0.3 per day at 25^{o}C. Depuration rates were not affected by salinity, pH, algae content, and turbidity. Mcleod et al. (McLeod et al., 2009) similarly found no significant reduction in the number of HAV in *C. gigas*. Furthermore, Nappier et al. (2010) showed that the proportion of *C. ariakensis* and *C. virginica* oysters with HAV RNA is time-independent until at least 29 days of depuration. These data suggest that the efficiency of depuration on the number of infectious HAV or HAV RNA in oysters and mussels is minimal

Table 3 Overview of quantitative data from the literature on bioaccumulation of viruses in shellfish

Shellfish	Virus	Method	Spike	Vol.	Temp	Conc L^{-1}	N	Max. contamination	Time	Reference
Crassostrea gigas	Polio	Culture		3.5 L	13	1.9×10^7 pfu	4-6	4.6×10^3 pfu.g^{-1}(46%-88%)	12h-48h	(Girolamo et al., 1975)
Ostrea lurida	Polio	Culture		3.5 L	13	1.9×10^7 pfu	10-12	8.6×10^3 pfu.g^{-1}	12h-48h	(Girolamo et al., 1975)
Crassostrea glomerata	Reo III	Absorb.	9×10^{10}	4 L	19	NR[†]	3	3.1×10^{10} (oyster tissue)	30-40h	(Bedford et al., 1978)
Mytilus edulis	Polio	Culture	NR	13 L	15-20	5.0×10^5-5.0×10^6	NR	$10^{5.5}$ pfu.g^{-1}	24h	(Power and Collins, 1989)
Mytilus chilensis	HAV	Culture	1.5×10^7 $TCID_{50}$	1 L	12	1.5×10^7 $TCID_{50}$	4	3.2×10^5 $TCID_{50}$ per mL[‡]	24h	(Enriquez et al., 1992)
Myltilus spp	Rota	Culture	10^7–10^8 MPNCU	4 L	21-23	8.0×10^6 MPNCU	40	1.1×10^4 MPUCU.g^{-1} (35%)	6h	(Abad et al., 1997b)
	Adeno	Culture	10^7–10^8 MPNCU	4 L	21-23	2.5×10^7 MPNCU	40	1.7×10^5 MPUCU.g^{-1} (25%)	6h	(Abad et al., 1997b)
	HAV	Culture	10^7–10^8 MPNCU	4 L	21-23	NR	40	(56%) data not given	6h	(Abad et al., 1997b)
	Polio 1	Culture	10^7–10^8 MPNCU	4 L	21-23	NR	40	(4%) data not given	6h	(Abad et al., 1997b)
Crassostrea gigas	RotaVLP	Absorb.	5×10^{14}	3000 L	22	1.7×10^{11}	100-120	1×10^{12} g^{-1}	24h	(Loisy et al., 2005)
	RotaVLP	Absorb.	5×10^{12}	3000 L	22	1.7×10^9	100-120	1×10^{10} g_{-1}	24h	(Loisy et al., 2005)
	RotaVLP	Absorb.	5×10^8	3000 L	22	1.7×10^5	100-120	1×10^6 g^{-1}	24h	(Loisy et al., 2005)
	RotaVLP	Absorb.	5×10^8	3000 L	22	1.7×10^5	100-120	1×10^6 g	24h	(Loisy et al., 2005)
Crassostrea gigas	NoV GII	qRT-PCR		50 L	10±2	2.77±0.59 Log_{10}	10	1.7×10^3 g^{-1} (SD=1.6×10^3)	72h	(Ueki et al., 2007)
	Fel. calici f4	qRT-PCR		50 L	10±2	2.87±0.6 Log_{10}	10	2.2×10^3.g^{-1} (SD=1.6×10^3)	72h	(Ueki et al., 2007)
Crassostrea virginica	mNoV	RT-PCR[**]		14 L	20-23	10^6 pfu	45	18 positive (dig. divert.)	24h	(Nappier et al., 2008)
	NoV GG1	RT-PCR		14 L	20-23	10^6 PCR units	45	7 positive (dig. divert.)	24h	(Nappier et al., 2008)
	HAV	RT-PCR[**]		14 L	20-23	10^6 pfu	45	8 positive (dig. divert.)	24h	(Nappier et al., 2008)
	Polio	RT-PCR[**]		14 L	20-23	10^6 pfu	45	2 positive (dig. divert.)	24h	(Nappier et al., 2008)
Crassostrea arakensis	mNoV	RT-PCR[**]		14 L	20-23	10^6 pfu	54	37 positive (dig. divert.)	24h	(Nappier et al., 2008)
	NoV GG1	RT-PCR		14 L	20-23	10^6 PCR units	54	30 positive (dig. divert.)	24h	(Nappier et al., 2008)
	HAV	RT-PCR[**]		14 L	20-23	10^6 pfu	54	27 positive (dig. divert.)	24h	(Nappier et al., 2008)
	Polio	RT-PCR[**]		14 L	20-23	10^6 pfu	54	1 positive (dig. divert.)	24h	(Nappier et al., 2008)

[†] NR: Not reported

[‡] per mL of mussel extract. Total of 8 mL extract analyzed

[**] Spike produced and analyzed by cell-culture; oysters analyzed by RT-PCR

and therefore does not need to be included in a quantitative risk assessment model.

In addition to quantitative data, presence/absence data on depuration of viruses have been reported (Table 4). These studies have demonstrated that depuration rates do vary between shellfish species (Nappier et al., 2008) and that individual oysters can harbour and retain up to three viruses simultaneously for four weeks after the initial exposure to virally contaminated water (Nappier et al., 2008). A longer-term purification process than depuration can also be applied, and is referred to as relaying (often more than ten days). Harvested shellfish are then transferred from a contaminated area to a clean area and laid on the ocean floor in racks. The only quantitative data identified in this review on the efficacy of relaying for the removal of viruses were reported by Loisy et al. (2005) using rotavirus virus-like particles as surrogates for viruses. In that study (see Table 5) approximately 70 days were required to achieve a 4 Log_{10} reduction in virus concentration. While relaying can provide effective removal of bacteria, very long durations of relaying would be required for efficient virus removal, and maintaining the integrity (free of subsequent viral contamination) of the relaying site for the required duration is a challenge.

2.3.5 Inactivation rate of HAV in shellfish

Under natural conditions inactivation of viruses in shellfish may occur at varying rates, depending on shellfish species and virus type (McLeod et al., 2009). To assess the level of virus inactivation, the ratio of infectious viral particles to the number of PCR amplifiable units was calculated in bioaccumulated pacific oysters (McLeod et al., 2009). Bioaccumulation of HAV resulted in a ratio of 0.66±0.06, indicating that most of the HAV in the oyster gut was still infectious. After a depuration period of 23 hours, a large proportion of HAV was still infectious with a ratio of 0.44±0.03.

Thorough cooking is the most effective method for inactivating virus contamination of food. However, for shellfish, high temperatures can affect the texture of the meat making it less palatable. In practice, cooking of shellfish is therefore often brief, and sufficient temperatures for virus inactivation may not be achieved. Bertrand et al. (Bertrand et al., 2012) estimated the temperature dependent inactivation rate for different viruses in 'simple' (i.e., media and water) and 'complex' (i.e., sewage, soil and mussels) matrices at temperatures below and above 50°C. The analyses included HAV and the results for complex matrices are relevant for estimating inactivation rates in shellfish. Using their results, the estimated time to first log_{10} unit reduction (TFL) of infectious HAV at 4°C, 20°C and 56°C was 76 (95% prediction interval: 6–928), 25 (2–302) and 0.003 (0.0002–0.04) days, respectively. The estimated TFL for HAV based on RT-PCR detection mounted to 0.007 (0.001–0.09) days for exposure to a temperature of 56°C. Estimates for temperatures <50°C were not reported. High pressure is an emerging process intervention to inactivate viruses in shellfish and to facilitate the shucking of oysters. Commercial processors use around 275 – 300 MPa of pressure for around three minutes to disinfect oysters. The advantage of the process is that the taste and texture of the shellfish are similar to the raw product, however with a partially cooked appearance. Calci et al. (2005) investigated the inactivation of HAV by high pressure treatment within oysters. Oysters that had been allowed to accumulate HAV were subsequently exposed to varying levels of pressure treatment. Similarly, Terio et al. (2010) investigated the impact of pressure treatment of mussels

contaminated with HAV. The results from those studies are summarized in Table 6. Both studies applied tissue culture assay since HAV inactivation by pressure does not appear to release the viral RNA molecule from the capsid (Kingsley et al., 2002). Using these data to estimate HAV decay with a monophasic decay model (for details on the model see Verhaelen et al., 2012), the inactivation rate was estimated at 0.0068 (95% interval: 0.0049 – 0.0091) per MPa pressure increase within the range of 300 to 400 MPa. There was no statistically significant difference between the different experiments and time of pressure applied. The HAV concentration after exposure to pressure P for up to five min can therefore be estimated from the initial HAV concentration, C_0, according to $C_p=C_0\times\text{Exp}[-0.0068(P-300)]$.

Table 4 Overview of quantitative data from the literature regarding elimination of viruses from shellfish during depuration

Shellfish	Location	Virus	Quantification by	Initial concentration	Max Log_{10} reduction	Time (hrs)	Tank details	Reference
Ostrea lurida	US	Poliovirus	Cell culture	3.4×10^3 pfu.g^{-1}	1.4	48	Stationary	(Girolamo et al., 1975)
Mytilus edulis	Ireland	Poliovirus	Cell culture	$10^{5.5}$ pfu.g^{-1}	1.86	52	Continuous flow	(Power and Collins, 1989)
		E. coli		$10^{3.7}$ cfu.g^{-1}	2.8	52		(Power and Collins, 1989)
		Coliphage Φ A1 – 5a		$10^{2.2}$ pfu.g^{-1}	2.16	52		(Power and Collins, 1989)
Mytilus chilensis	Chile	HAV	Cell culture	~ 10^5 $TCID_{50}$	1	168	Stationary	(Enriquez et al., 1992)
		Poliovirus		~10^7 $TCID_{50}$	>5	3	Stationary	(Enriquez et al., 1992)
Myltilus spp	Spain	Rotavirus	Cell culture – 96 well – microtiter plates	1.1×10^4 MPUCU.g^{-1}	1.52	96	Continuous flow	(Abad et al., 1997b)
		Human Adenovirus type 40		1.7×10^5 MPUCU.g^{-1}	2.74	96	Continuous flow	(Abad et al., 1997a)
		HAV		data not given	1.89	96	Continuous flow	(Abad et al., 1997a)
		Poliovirus 1		data not given	3	48	Continuous flow	(Abad et al., 1997a)
Crassostrea gigas	France	Rotavirus VLP	Spectrophotometer	1×10^{12} g dissected tissue from ten oysters	1.5	168	Continuous flow	(Loisy et al., 2005)
				1×10^{10} g dissected tissue from ten oysters	1	168		(Loisy et al., 2005)
				1×10^6 g dissected tissue from ten oysters	1	168		(Loisy et al., 2005)
Crassostrea gigas (Pacific oysters)	Japan	Norovirus GII	qRT-PCR	Average 1.7×10^3 copies.g^{-1} (SD=1.6×10^3)	0	240	Continuous flow	(Ueki et al., 2007)
		Feline Calicivirus f4	qRT-PCR	Average 2.2×10^3 copies.g^{-1} (SD=1.6×10^3)	3	72	Continuous flow	(Ueki et al., 2007)

*Spike produced and analysed by cell-culture; oysters analysed by RT-PCR; NR: Not reported

Table 5 Reductions of numbers of VLPs in oysters under relaying conditions[a] (reproduced from (Loisy et al., 2005))

No. of days of relaying	Log_{10} of VLP concentration in oyster tissue for:		
	Expt A	Expt B	Expt C
0	10.5	9	5
7	10	9	5
14	10	8	4.5
21	8.5	8	ND
30	ND	ND	4
37	ND	ND	4
41	7.5	6.5	<4
49	7	6	ND
70	ND	5	ND
82	5	<4	ND
89	<4	ND	ND

[a]After 1 week of depuration, oysters were placed on the shore under the influence of tides for a natural relaying. Samples were collected and analysed by ELISA In duplicates. ND: analysis not done.

2.3.6 HAV in harvested shellfish

A large number of studies have been undertaken to assess the prevalence of HAV in mussels and oysters in Europe (see Table 7). An important aspect to consider in comparing the percentages is the different analytical methods that were used in the presented studies. These differences will lead to different estimates for the concentration when applied to the same samples. These differences are caused by different sensitivities of methods, different detection limits, and in case of real-time RT-PCR possibly different quantification methods. Furthermore, if suboptimal amplification efficiencies, e.g. due to inhibition, are neglected, then virus concentrations will be underestimated. Approaches to tackle such aspects in quantification were recently published (D'Agostino et al., 2011; Lees, 2010). The subsequent quantification ideally comprises a level of variation or uncertainty, which is currently not reported. Such information is essential to include in quantitative microbiological risk assessment and can be obtained using the raw data from the experiments. An example of including uncertainty in concentration estimates from real-time RT-PCR can be found in Verhaelen et al (2012). In this approach, the uncertainty of the standard curve is included in the uncertainty of the subsequent count derived from the Ct-value, which is more representative of the uncertainty.

Table 6. Reported efficiency of pressure treatment for virus inactivation

			Pressure applied (MPa)						
		Time (m)	0	300	325	350	375	400	Reference
Crassostrea virginica	Log_{10} pfu reduction	1	5.8	5.6 0.2	5.0 0.8	4.5 1.3	3.5 2.3	2.7 3.2	(Calci et al., 2005)
M. edulis	Log_{10} pfu reduction	5	6.2	6.0 0.1	5.5 0.7	4.4 1.7	3.7 2.5	3.3 2.9	(Terio et al., 2010)
M. gallo-provincialis	Log_{10} pfu reduction	5	5.6	4.8 0.8	4.6 1.0	3.5 2.1	2.9 2.7	2.1 3.6	(Terio et al., 2010)

Table 7 Summarised literature data for prevalence of HAV RNA in oysters and mussels in Europe

Country of sampling	Sampling Year	Shellfish type	No. of specimen	No. of pools tested	HAV Pos.	Reference
Retail						
Switzerland	2001-2002	*C. gigas/O. edulis*	435	87 of 5 spec.	0	(Beuret et al., 2003)
Switzerland	1996	*C. angulata* and *O. edulis*	3	ind.	0	(Hafliger et al., 1997)
Switzerland	1996	*M. edulis*	3	Ind.	0	(Hafliger et al., 1997)
Italy or unknown	1999-2000	*M. galloprovincialis*	NR*	100 of 10 g	23	(Chironna et al., 2002)
Italy	NR	*M. edulis*	89	ind.	30	(De Medici et al., 2001)
Spain	2010	*M. galloprovincialis*	51	ind.	0	(Diez-Valcarce et al., 2012)
Greece	2010	*M. galloprovincialis*	51	ind.	0	(Diez-Valcarce et al., 2012)
Finland	2010	*M. edulis*	51	ind.	0	(Diez-Valcarce et al., 2012)
Class A growing area						
Spain	1999	*M. edulis*	NR	2	0	(Muniain-Mujika et al., 2003)
Spain	1999	*C. gigas*	NR	5	0	(Muniain-Mujika et al., 2003)
Italy	2005-2006	*M. galloprovincialis*	120	24 of 5 spec.	0	(Suffredini et al., 2008)
The Netherlands	2000-2001	*C. gigas*	NR	28 of 5/6 spec.	0	(Lodder-Verschoor et al., 2005)
The Netherlands	2000-2001	*O. edulis*	NR	10 of 5/6 spec.	0	(Lodder-Verschoor et al., 2005)
Class B growing area						
France	1995-1998	*C. gigas*	NR	108	0	(Le Guyader et al., 2000)
Spain	1998-1999	*M. edulis*	NR	35	8	(Muniain-Mujika et al., 2003)
Spain	1999	*C. gigas*	NR	10	2	(Muniain-Mujika et al., 2003)
Italy	2005-2006	*M. galloprovincialis*	240	48 of 5 spec.	0	(Suffredini et al., 2008)
Italy	2003-2004	*M. galloprovincialis*	53	ind.	2	(Croci et al., 2007)
Italy	2003-2004	*Ostrea spp.*	8	ind.	0	(Croci et al., 2007)
Italy	NR	*Mytilus*	36	ind.	14	(Croci et al., 2000)
Spain	2005	*M. galloprovincialis*	NR	24 of ≥10 spec.	0	(Vilarino et al., 2009)
Portugal	2008-2009	*Mytilus* spp.	NR	9 of ≥20 g DT	5	(Mesquita et al., 2011)
Class C or D growing area						
France	1995-1998	*M. galloprovincialis*	NR	73	6	(Le Guyader et al., 2000)
Spain	2005	*M. galloprovincialis*	NR	12 of ≥10 spec.	0	(Vilarino et al., 2009)
Portugal	2008-2009	*Mytilus* spp.	NR	5 of ≥20 g DT	4	(Mesquita et al., 2011)
Portugal	2008-2009	*O. edulis*	NR	7 of ≥20 g DT	1	(Mesquita et al., 2011)
Various or unspecified						
France	1992-1993	*M. edulis*	10	ind.	0	(Le Guyader et al., 1994)
France	1990-1991	*M. edulis*	NR	19	12	(Le Guyader et al., 1993)

* NR: not reported

Table 7 Continued

Country of sampling	Sampling Year	Shellfish type	No. of specimen	No. of pools tested	HAV Pos.	Reference
France	1990-1991	*M. edulis*	NR*	17	13	(Le Guyader et al., 1993)
France	1990-1991	*M. edulis*	NR	18	12	(Le Guyader et al., 1993)
France	1992-1993	*C. gigas*	8	ind.	3	(Le Guyader et al., 1994)
Spain	1999-2000	*M. galloprovincialis*	NR	20 of 10 g	0	(Chironna et al., 2002)
Spain	1999	*M. galloprovicialis*	NR	6	4	(Muniain-Mujika et al., 2003)
Spain	2000-2001	'Oysters and mussels'	104	ind.	3	(Formiga-Cruz et al., 2002)
Spain	2000	*M. galloprovincialis* (wild)	NR	78	23	(Romalde et al., 2002)
Spain	2000	*M. galloprovincialis* (raft cultured)	NR	58	15	(Romalde et al., 2002)
Italy (dep.)	1999-2000	*M. galloprovincialis*	NR	10 of 10 g	2	(Chironna et al., 2002)
Italy (undep.)	1999-2000	*M. galloprovincialis*	NR	10 of 10 g	3	(Chironna et al., 2002)
Italy	NR	*M. galloprovincialis*	NR	75	4	(Macaluso et al., 2006)
Italy	NR	*C. gigas*	NR	22	1	(Macaluso et al., 2006)
Greece	2000-2001	'Oysters and mussels'	144	ind.	6	(Formiga-Cruz et al., 2002)
Greece (dep.)	1999-2000	*M. galloprovincialis*	NR	80 of 10 g	8	(Chironna et al., 2002)
Greece (undep.)	1999-2000	*M. galloprovincialis*	NR	70 of 10 g	17	(Chironna et al., 2002)
UK	2000-2001	'Oysters and mussels'	173	ind.	17	(Formiga-Cruz et al., 2002)
UK	NR	*O. edulis*	NR	3 of 9.4 g	0	(Lees et al., 1995)
UK	NR	*M. edulis*	NR	6 of 9.4 g	0	(Lees et al., 1995)
UK	NR	*C. gigas*	NR	1 of 9.4 g	0	(Lees et al., 1995)
The Netherlands	2000-2001	*C. gigas*	NR	28 of 5/6	0	(Lodder-Verschoor et al., 2005)
Sweden	2000-2001	'Oysters and mussels'	54	ind.	0	(Formiga-Cruz et al., 2002)

* NR: not reported

2.3.7 Consumption of shellfish

Previous studies reported that a consumption event consists of approximately eight to ten oysters (Lowther et al., 2010). A web survey of 25 menus from Dutch restaurants showed approximately similar results, with a medium of six oysters with a range from 5 to 12 (unpublished data).

A national food consumption survey was recently conducted in the Netherlands, where people between 6 and 79 years of age were asked to register food intake during two days. Data for specific food items were not yet available when this report was finished, but are expected to become available in 2013.

3 Hepatitis E virus in pork

3.1 Introduction

Hepatitis E virus (HEV) is the aetiological agent of non-HAV enterically transmitted hepatitis recently classified in the genus Hepevirus of the family Hepeviridae. HEV has at least two distinct epidemiological profiles: (1) large outbreaks and epidemics in developing countries, usually caused by HEV genotype 1, resulting in high morbidity and mortality among pregnant women and young children, and (2) very few symptomatic cases of HEV genotype 3, most cases without symptoms or clear source(s) of infection, but frequent seroreactivity in 5%-21% of asymptomatic persons in developed countries. Though the first is largely considered to be the result of a water-borne infection, the latter is suspected to result from foodborne zoonoses.

It is generally accepted that hepatitis E is mostly self-limited and rarely progresses to chronicity. HEV infections have a higher mortality in pregnant women compared to non-pregnant individuals, where the disease condition is accentuated with the development of fulminant liver disease. Clinical manifestations of HEV infection vary widely from asymptomatic infection to uncomplicated acute viral hepatitis and fulminant hepatic failure. No anti-HEV treatment exists but two subunit vaccines containing recombinant truncated capsid proteins of HEV have been shown to be highly effective in preventing the disease (Zhu et al., 2010); however, these are not yet commercially available (Kamili, 2011).

HEV is a pathogenic virus for humans, but susceptibility to the virus can be found among a whole range of animals, including domestic pigs (Meng, 2010). Domestic pigs are most abundantly examined from all animal species, and found to be massively infected.

In Europe, no evidence for one main transmission route of HEV infection or risk factor for hepatitis E could be identified; however, zoonotic transmission seems likely (Lewis et al., 2010). HEV has been detected in multiple animal and environmental sources with the highest prevalence in domestic pigs (Rutjes et al. 2009). Pig products such as pig livers were shown to contain HEV (Bouwknegt et al., 2007). Also 20/39 muscle samples from pigs contact-infected by HEV in an experiment contained HEV RNA suggesting possible foodborne transmission through pork meat consumption (Bouwknegt et al., 2009b). Contamination of meat is likely a consequence of viremia, suggesting that meat obtained from pigs in the acute phase of infection at slaughter can be contaminated by HEV (Bouwknegt et al., 2009b). Recently, the consumption of raw pig liver sausages was implicated in hepatitis E disease in patients in France (Colson et al., 2010). Previously, HEV infection was associated with the consumption of uncooked deer meat (Tei et al., 2003) substantiated with a matched case-control study (Tei et al., 2004). HEV was also detected in a proportion of the Dutch deer and wild boar(Rutjes et al., 2010; Rutjes et al., 2009b). Consumption of uncooked meat, still practised worldwide, is generally considered a risk for infection and disease, now shown for HEV. This risk may be averted by cooking (Emerson et al., 2005; Feagins et al., 2008), but risk management options seem to be limited. To be able to estimate public health risks from consumption of contaminated meat (products) by QMRA, quantitative

data on virus concentrations in meat need to be collected. Therefore, quantitative data on HEV in the different phases of the meat production chain from HEV in farms to HEV in meat on the market were gathered for risk assessments with the purpose of informing risk management.

3.2 Components of the conceptual risk assessment model

Hepatitis E virus can infect a broad range of animals and thereby provide multiple possible sources of zoonotic infection from consumption of meat and meat products. Domestic pigs have been studied most frequently of all animal species and high prevalence rates of over 50% are common on European pig farms (e.g. Rutjes et al., 2007). Furthermore, HEV spreads among pigs, and therefore pigs have the potential to be an animal reservoir (Bouwknegt et al., 2008b). Therefore the exposure to HEV will focus on pork in this QVRP.

Exposure of humans to porcine HEV can occur via direct contact with pigs (Bouwknegt et al., 2008a; Withers et al., 2002), via indirect transmission (e.g., associated with water due to HEV runoff from land into surface waters after fertilization), or via consumption of pork products (Figure 2). This QVRP for HEV focuses on the latter route. Pork and pork products can become contaminated through two routes:

- intrinsic contamination because a pig is systemically infected with HEV;
- extrinsic contamination due to cross contamination with faeces, bile or blood.

Both routes will be considered in the conceptual model detailed below.

As a result of the intrinsic HEV infection of pigs, pork meat (products) may be contaminated with HEV. Consumption of these products may lead to HEV exposure, and possibly to HEV infection and hepatitis E. The exposure of humans can be quantified as the ingested dose per consumption event, which consists of the sum of the infectious HEV per gram of final pork product and the amount of meat consumed. Sparse meat consumption data are gathered from the Dutch Food Consumption Surveys, which can be used as baseline scenarios in the risk analysis. The number of infectious HEV in the final pork product depends on the number of HEV per gram of tissue, the dilution of that amount due to mixing with HEV-negative ingredients during processing and HEV-inactivation during processing and preparation prior to consumption.

The exposure related to extrinsic contamination of pork products similarly depends on the sum of the infectious HEV per gram of final pork product and the amount of product consumed. The amount of infectious HEV per gram of product depends on the HEV concentration in the contaminating substance (e.g., faeces), on the amount of substance transferred to the pork product and on processing factors that dilute or remove infectious HEV from the pork product (e.g., washing-off or heat inactivation).

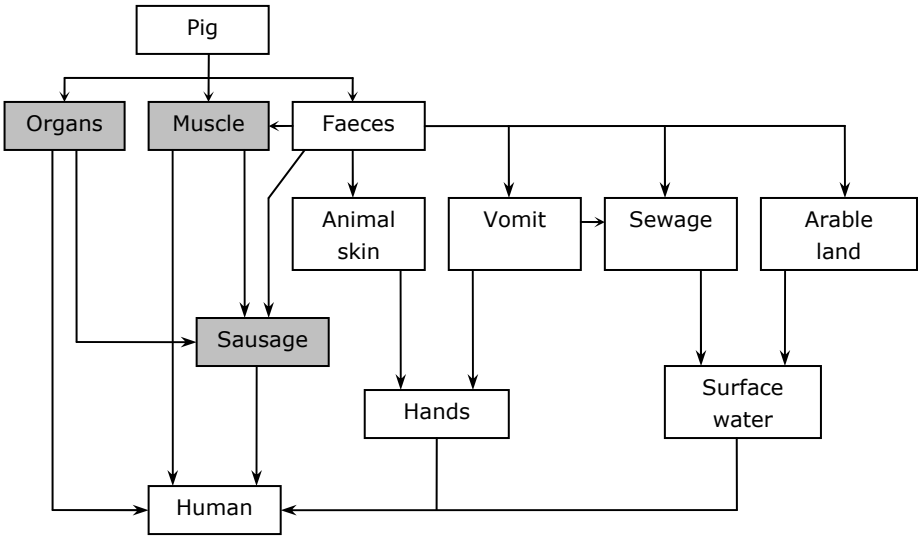

Figure 2 Conceptual exposure pathway with the most predominant routes for HEV contaminated pork meat consumption. Blood is not included explicitly in this scheme, but is considered a source for contamination of organs and meat.

3.3 Component quantification

3.3.1 Prevalence of HEV infection in pigs at slaughter

An important determinant for the risk associated with HEV infection due to pork consumption is the prevalence of HEV infection in pigs at slaughter, when pigs are about 26 weeks of age. This prevalence depends on the epidemiology of HEV infections in pigs. At the age of two weeks, pigs can become infected and excrete HEV faecally (Fernandez Barredo et al., 2006; LeBlanc et al., 2007). The highest prevalence of faecal HEV excretion is observed among pigs from ten weeks of age until three months (LeBlanc et al., 2007; Nakai et al., 2006; Seminati et al., 2008). This finding might indicate that HEV infection occurs soon after the onset of fattening, when pigs are about ten weeks of age. During the fattening period, the prevalence of infected pigs can reach up to 100% (Takahashi et al., 2003). The reported HEV prevalence at slaughter is lower again due to successful clearance of the virus by pigs during the fattening period. Table 8 lists an overview of HEV RNA prevalence at pigs of slaughter age, collected either at pig farms just prior to slaughter, or at slaughterhouses. In the European FP7 project 'VITAL', individual faeces, muscle and liver samples were collected from pigs in slaughterhouses in four different countries to estimate the number of infected pigs at slaughter (Di Bartolo et al., 2012). The results are reproduced in Table 9. The data were combined to estimate the scale and shape parameter of a Beta(α, β) distribution using beta-regression (Espinheira et al., 2008; Ferrari and Cribari-Neto, 2004). The values for the parameter of the beta distribution were estimated as part of this QVRP using Mathematica v8 (Wolfram Research Inc., USA). The estimated Beta-distribution for the variation of proportion of pigs infected with HEV at slaughter mounted to Beta(0.93, 3.6), with mean 0.20 and 95% interval from 0.01 to 0.63.

3.3.2 Fraction of infected pigs that are viremic

The contamination of meat and organs, other than the liver, is most likely the result of HEV being present in the bloodstream (i.e., when pigs are viremic), because HEV does not replicate in organs other than liver and possibly the intestines (Williams et al., 2001). HEV detection in faeces, (i.e., when pigs are

excretic) is generally used to assess whether a pig is currently infected or not (presence of antibodies in serum is a measure of past infection). Only a fraction of the pigs that are found to excrete HEV faecally (here referred to as 'excretic pigs') are in the viremic state of infection. The period during which HEV can be detected in faeces usually spans the period of viremia (Bouwknegt et al., 2009b). When the subpopulation of examined pigs is selected randomly at or close to slaughter, then the average fraction of viremic pigs among the excretic pigs is approximately proportional to the ratio between the length of the viremic period and the excretic period. The length of the excretic period was estimated for contact-infected pigs to be 23.3 days (95% interval: 18.7 – 27.9) and the viremic period 10.5 days (8.1 – 13) (Bouwknegt et al., 2009b). The ratio between the two is therefore 10.5/23.3=0.45 (95% interval obtained by MC simulation of the fraction with 10^6 iterations: 0.33 – 0.61). Thus, on average 45% of the excretic pigs are estimated to be viremic.

Table 8 The prevalence of HEV RNA in pigs of slaughter age sampled at farms or at slaughterhouses, based on different sample types

Country	No. of farms	No. of pigs	HEV positive	Remark	Reference
Faeces					
Japan	3	36	3 (8%)	sampled on farm; 6 months old	(Nakai et al., 2006)
Canada	1	51	21 (41%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2007)
Spain	NR*	28	2 (7%)	sampled on farm	(Fernandez Barredo et al., 2006)
Italy	NR	20	13 (65%)	3-4 months old; sampled at slaughter	(Di Bartolo et al., 2012)
Italy	NR	28	3 (11%)	9-10 months old; sampled at slaughter	(Di Bartolo et al., 2012)
Canada	1	43	6 (14%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2010)
Spain	5	23	0 (0%)	sampled at slaughter	(Casas et al., 2011)
Italy	NR	150	11 (7%)	9-12 months old; sampled at slaughter	(Di Martino et al., 2010)
Nether-lands	10	50	7 (14%)	five pigs sampled per farm, at the slaughterhouse; three of ten farms positive	(Rutjes et al., 2009a)
Blood					
Canada	1	51	6 (12%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2007)
Canada	1	43	1 (2%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2010)
Bile					
Italy	NR	19	13 (68%)	3-4 months old; at slaughterhouse	(Di Bartolo et al., 2012)
Italy	NR	26	10 (38%)	9-10 months old; sampled at slaughter	(Di Bartolo et al., 2012)
Brazil	NR	115	11 (10%)	sampled at three slaughterhouses	(Dos Santos et al., 2011)
Canada	1	43	8 (19%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2010)
Spain	6	80	5 (6%)	sampled at slaughter	(Casas et al., 2011)
Liver					
Japan	NR	363	7 (2%)	sampled at retail	(Yazaki et al., 2003)
Nether-lands	NR	62	4 (6%)	sampled at retail	(Bouwknegt et al., 2007)
United States	NR	127	14 (11%)	sampled at retail	(Feagins et al., 2007)
United Kingdom	NR	80	0 (0%)	sampled at retail	(Banks et al., 2007)
Italy	NR	20	6 (30%)	3-4 months old; sampled at slaughter	(Di Bartolo et al., 2012)
Italy	NR	28	4 (14%)	9-10 months old; sampled at slaughter	(Di Bartolo et al., 2012)
Canada	1	43	9 (21%)	simulated commercial farm; sampled at slaughter	(LeBlanc et al., 2010)
France	186	3715	128 (4%)	prevalence estimate accounts for survey design	(Rose et al., 2011)
China	NR	114	4 (4%)	sampled at slaughter	(Li et al., 2009)
Spain	6	96	6 (6%)	sampled at slaughter	(Casas et al., 2011)

* NR: not reported

Table 9 Prevalence of HEV RNA (number positive/number examined) at slaughterhouses in four different European countries (Di Bartolo et al., 2012)

Country	Faeces	Liver	Meat	Pigs
Country A	1/40 (3%)	2/40 (5%)	1/40 (3%)	3/40 (8%)
Country B	14/34 (41%)	2/33 (6%)	2/33 (6%)	17/34 (50%)
Country C	15/39 (38%)	1/39 (3%)	0/40	15/40 (38%)
Country D	5/40 (13%)	1/40 (3%)	n.d.*	6/40 (15%)

* n.d.: no data

3.3.3 Concentration of HEV in pig meat at slaughter

HEV particles are detected in samples by (RT-)PCR, which is aimed to detect specific HEV RNA from all RNA present in the sample. This method detects HEV RNA from infectious and non-infectious virus particles. This aspect is important for QMRA for viruses, because the lack of this distinction may result in overestimation of the infection risk. Infectivity, posed by infectious HEV only, is the determinant of human infection after ingestion and thus of the infection risk. If the total HEV RNA is enumerated instead of the RNA from infectious HEV particles, then the estimated infection risk will be overestimated. Distinction between the inactivated and the infectious form of HEV cannot be made since a cell culture method for detection of infectious HEV does not exist to date. From qPCR data, assumptions can be made for QMRA regarding the fraction of infectious HEV among all HEV detected, as described previously (Bouwknegt et al., 2011).

A second difficulty in HEV quantification by RT-PCR concerns the actual absolute quantification of the initial RNA count. Two approaches for quantification exist: using a real-time RT-PCR system and relate the signal (i.e., Ct value) for samples to that of a standard curve with a presumed known genome count of a representative standard. Issues that affect the accurateness of the estimation includes amongst others the representativeness of a control sample for HEV, differences in amplification efficiency due to matrix differences (e.g. water versus faeces) and the method used for quantification of the standard. The latter is also an estimate based on some measure, such as OD, but its accurateness is poorly known.

A second approach for quantification involves an approach similar to most probable number estimation (Cochran, 1950). Based on the presence/absence detection of HEV in serial tenfold dilutions of RNA, and the assumption of homogeneous mixing, an estimate of the number of so-called PCR detectable units (PDU) is obtained. The number of HEV genomes reflected by a PDU, however, is indeterminable. In the most ideal situation, a single PDU consist of one HEV particle, but a PDU can also represent an unknown multiple of one particle. The true number of HEV particles reflected by a PDU depends on the performance of nucleic acid extraction and PCR assay, and on the test characteristics such as sensitivity and specificity. In conclusion, both methods for quantification have their advantages and disadvantages, and the performance of one method over the other is unknown.

The MPN approach for quantification was employed in 'VITAL' and the three positive meat samples from Table 10 were positive at a level of 5×10^4 (95% interval: 8×10^3–4×10^5) PDU, 50 (1–230) PDU and 700 (40–3200) PDU (Bouwknegt et al., in preparation). Furthermore, meat was found positive in 20 of 39 muscle samples collected from experimentally infected pigs (Bouwknegt et

al., 2009b). The concentration of HEV in these samples was, however, not quantified. No other data on HEV concentrations in meat were found in the literature.

3.3.4 Natural inactivation rate of HEV

The infectivity of viruses is generally assessed experimentally by exposing in vitro cells with the required receptor(s) to HEV. This method is referred to as cell culture. Infectious viruses will intrude cells and replicate. This intrusion or replication is thus a measure for infectivity, and can be measured by visualization of the infected cells using for instance immunostaining or detection of an increase in virus number. For HEV, however, no cell culture system is available that is sufficiently sensitive and practical to conduct inactivation experiments with HEV. Several studies do report successful propagation of HEV on specific cell lines or in specific culturing techniques (Berto et al., 2012; Rogee et al., 2012; Shukla et al., 2012). Further development of those systems might yield sensitive and practical cell culture systems for HEV to study its stability.

Some information on the stability of HEV is available from the epidemiological studies that have been conducted. For instance, HEV of genotype 1 has been attributed to outbreaks that can last several years. Water is often the implicated source for exposure of humans to HEV (Emerson et al., 2005). Possible explanations for prolonged outbreaks are that HEV-susceptible individuals enter the population continuously, and/or newly excreted HEV adds to the environmental HEV source continuously. In all cases, however, HEV is unlikely to be inactivated at a large rate upon excretion by infected individuals.

3.3.5 Heat inactivation of HEV

As indicated above, the effect of temperature on the infectivity of viruses can be examined with a culture system. Such a cell culture system, however, has not been developed for HEV and robust heat inactivation studies are therefore not performed to date.

Emerson et al. (Emerson et al., 2005) showed in a cell infection experiment that the susceptibility of HEV to heat differed between HEV strains, where some were inactivated nearly completely when maintained at 56^{o}C for 1 h (a temperature-

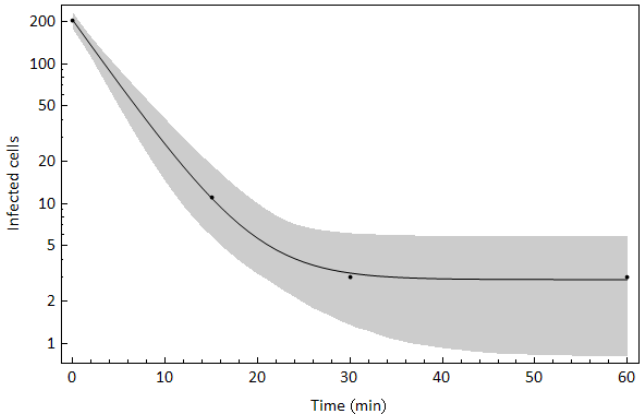

Figure 3 Inactivation curve for HEV, based on data from Emerson et al. (2005) These experiments showed, for HEV GT1 strain, that the initial infectivity decay is large, but is minimal during the second phase.

proxy for moderate heating of food), whereas for others 20% remained infective after being maintained at 60°C. Figure 3 shows a preliminary analysis of the decay of HEV genotype 1 (Akluj strain) that was observed by Emerson et al (2005). Although the dataset is very limited, the results suggest that a thermostable fraction of HEV may exist. Especially this fraction could be the main determinant for the public health risk. Similarly to observations by Emerson et al. (2005), Feagins et al. (2008) showed in an infection experiment with pigs that heating HEV-contaminated pork liver at 56°C was ineffective in reaching complete inactivation, whereas boiling or stir frying at 191°C for five minutes prevented pigs from becoming infected. Tanaka et al. (2007) demonstrated that the infectivity of HEV genotype 3 was not affected by a heat treatment at 25 °C and 56 °C for 30 minutes, whereas heating at 70 °C and 95 °C for ten minutes and for one minute, respectively, prevented the replication of the virus.

3.3.6 Other inactivation rates

For pork products the processing possibilities are much more complex compared to foods with a simpler production process, such as lettuce heads and raspberries. Depending on the final product, HEV will be challenged by e.g. desiccation, high salinity, smoking, and fermentation conditions. These processes could promote HEV inactivation, and thereby decrease its public health burden, but quantitative data on the effect of these conditions on HEV infectivity are lacking. Therefore, these processes cannot be included in a quantitative viral risk assessment to date.

3.3.7 Consumption of pork meat

A case control study that was done in the Netherlands in 2002-2003 to study risk factors for *Salmonella* and Campylobacter infections report that about 80% of 2452 interviewees (matched to cases by age, gender and region) consumed pork meat at least once in the seven days prior to completing the questionnaire (Doorduyn et al., 2010). Extrapolating this figure to an annual number of pork consumption, indicates that at least 10^8 pork consumption events per year exist. Unfortunately, no data on the type of pork meat are known. Data from a population-based cohort study from 1998-1999 showed that about 3% of the population consumes un(der)cooked pork (Van Duynhoven, personal communication). In that case at least 10^6 consumption event per year would

involve moderately or raw pork meat. This figure might be used as a starting value for pork meat consumption in general in the absence of more robust data.

As reported for consumption of shellfish in paragraph 2.3.7, a national food consumption survey was recently conducted in the Netherlands, where people between 6 and 79 years of age were asked to register food intake during two days. Data for pork consumption were not yet available when this report was finished, but are expected to become available in 2013.

3.3.8 Dose-response model

A dose response model for hepatitis E virus infections in humans is not yet available. Ethical arguments hamper volunteer exposure experiments, while the incubation period of up to ten weeks hampers the collection of relevant data from outbreaks to construct a dose-response model. A dose response model has been developed for intravenous inoculation of pigs, and statistically adjusted to reflect oral ingestion of HEV (Figure 4, reproduced from Bouwknegt et al. (2011)). This exponential model involves an estimated infectivity per HEV particles (r) of 1.3×10^{-2}. This model was adjusted to represent the probability of infection following oral inoculation by reducing the probability of infection per HEV particle by a factor of 10^4. This factor was based on the observation that faecal-orally transmitted HEV is at least 10,000 times less infective than intravenously inoculated (Kasorndorkbua et al., 2002). The probability of infection (P_{inf}) per infectious HEV particle given a certain ingested HEV-dose (D) is modelled by

$$P_{inf} = 1 - e^{-r \cdot D}$$

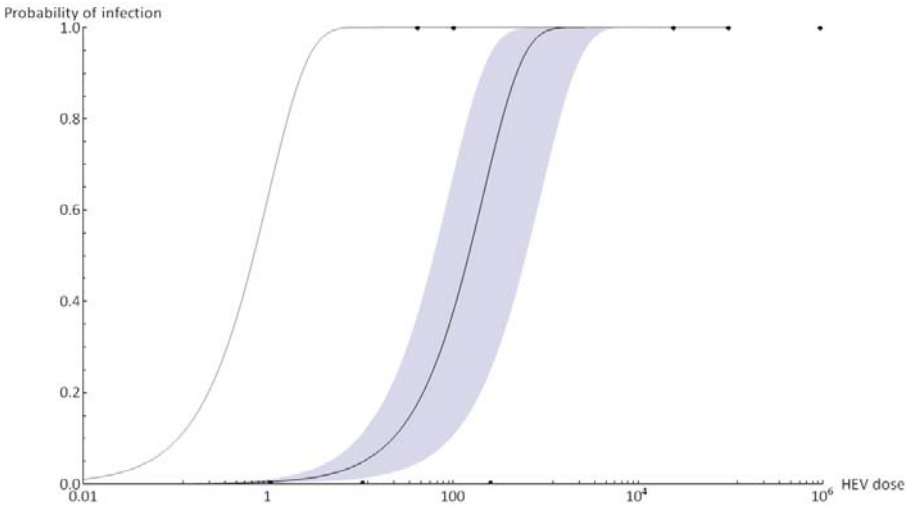

Figure 4 The dose-response relationship for intravenous inoculation of HEV in pigs. The black solid line represents the mean probability, the grey-shaded area the corresponding 95% confidence interval. The thin solid line represents the maximum probability of infection (i.e., when the probability of infection per HEV particle would be 1) and the dots represent the observed data. The x-axis (administered HEV dose) is on a log-scale. For oral ingestion of HEV, the curve is expected to shift at least four orders of magnitude to the right (as explained in paragraph 3.3.8).

This model might be used as a candidate-model for prediction of human infection risks given an estimated exposure dose. The requirement for validity of the risks for humans is that the pig needs to be a proper animal model for humans regarding HEV infection.

4 Norovirus on fresh produce

4.1 Introduction

Noroviruses (NoVs) are a major cause of viral gastroenteritis worldwide. They belong to the family of the Caliciviridae, are 28–35 nm in size, are non-enveloped viruses and contain a positive sense, single stranded RNA genome of approximately 7.6 kb (Green et al., 2002). The norovirus genus is divided into five genogroups (genogroup GGI to GGV). The diversity of norovirus variants increases continually due to the generation of new variants, and variants within GII.4 have predominated in Europe in the past five years (Hohne and Schreier, 2004; Kroneman et al., 2008; Lindell et al., 2005; Lopman et al., 2004; Maunula and Von Bonsdorff, 2005; Reuter et al., 2005). Most human pathogenic noroviruses cluster within genogroup I (GGI) and genogroup II (GGII). No zoonotic potential has been demonstrated for these two genogroups, and these are therefore considered to be solely prevalent in human.

Although typically a self-limited disease characterized by non-bloody diarrhea and vomiting, norovirus gastroenteritis can cause significant morbidity and mortality among children, the elderly, and the immunocompromised (Koo et al., 2010). In the Netherlands, the virus causes several hundreds of hospitalizations of children and elderly and approximately 20 deaths per year (Mattner et al., 2006; Patel et al., 2008; van Asten et al., 2011). Noroviruses may be transmitted from person to person or via other faecal-oral and fomite transmission routes involving the environment. And noroviruses are the leading cause of foodborne disease outbreaks worldwide. Recent investigations have identified fruits and vegetables as the source of many foodborne disease outbreaks with noroviruses as the leading causative agent (Berger et al., 2010).

Human noroviruses are pathogenic to humans only; no animal reservoir has been established to date (Bank-Wolf et al., 2010). Besides random mutations that occur during viral replication, the great heterogeneity observed among noroviruses is also due to intra and inter-genotypic recombination events between strains (Lindesmith et al., 2011). Some of these new variants or new recombinants are frequently associated with new epidemic waves of gastroenteritis due to the ability to escape herd immunity (Bull and White, 2011). The infectivity of norovirus is estimated to be high, with an estimated infection probability of 0.5 per virus particle based on human feeding trials (Teunis et al., 2008). The infection often progresses to a symptomatic disease, which is mostly self-limiting and rehydration therapy is usually sufficient for recovery. Vaccines based on norovirus capsid protein virus-like particles showed promising results for prevention of infection and may become widely available through transgenic expression in plants (Koo et al., 2010).

Norovirus outbreaks are commonly caused by consumption of contaminated foods (Verhoef et al., 2010). Such foods can become contaminated throughout the food production process, at production, processing or during food preparation by caterers and in homes. Fresh produce became a leading vehicle in the transmission of NoV via food with foodborne outbreaks associated with raspberries and lettuce (Doyle and Erickson, 2008). Such fresh produce are of special interest for public health, because these are generally consumed raw and are not treated with virucidal processes such as heating. The number of infectious viruses is therefore not reduced prior to consumption, as is the case

for instance for *Campylobacter* on chicken fillet. Therefore, this chapter of the quantitative risk profile for QVRA for norovirus will focus on the fresh produce salad vegetables and raspberries.

4.2 Components of the conceptual risk assessment model

As for the quantitative risk assessments for HAV and HEV described in Chapters 2 and 3, the dose of NoV to which humans are exposed consist of the number of NoV ingested per event of interest (e.g., a consumption event). The dose therefore depends on the number of infectious NoV ingested per portion of lettuce or per raspberry, and the number of portions or berries consumed.

As indicated in Figure 5, the eventual number of NoV per portion of produce can be the results of contamination from different routes. Firstly, hands of people that touch the product can be contaminated and introduce viruses into a batch of produce and contaminate a number of units per batch. These people can be for instance harvesters, food handlers at processing and retail, customers in retail stores that touch-to-judge produce, and the food preparers. Secondly, a batch of produce (and units therein) can become contaminated extrinsically due to contaminated irrigation water and due to splash dispersal of soil-bound viruses during irrigation or rainfall and use of pesticides reconstituted from surface water (Verhaelen et al., 2013b). The soil may become contaminated by viruses from irrigation and by intended or unintended fertilization (e.g. by harvesters that lack access to latrines). Thirdly, viruses might contaminate produce intrinsically by co-entering the produce with water and/or nutrients through the roots. Fourthly, any rinsing water used during processing of harvested produce can contaminate a batch. This contamination can consist of new introduction of viruses to the batch and its units when contaminated water is used. And by reusing rinsing water for multiple units and batches, previously washed contaminated produce can indirectly cross-contaminate other produce. Also fifthly utensils such as knives used during processing can be a source of virus introduction into a batch when improperly cleaned, or a source of cross-contamination within a batch of produce when contaminated produce has been processed previously.

4.3 Component quantification

4.3.1 Norovirus concentration in irrigation water

Abstraction from surface waters are the main source for irrigation in Greece (80%), Spain (68%), France (80%), Germany (75%), the UK and Ireland, however, in Denmark, Sweden, the Netherlands, Austria and Portugal the source of irrigation water is mainly groundwater (Baldock et al., 2000). The main sources of human noroviruses in surface and groundwater that is used for irrigation are sewage discharges and faecal wastes of humans. Noroviruses are frequently found in rivers and lakes, but also in groundwater and well water (Borchardt et al., 2003; Lodder and de Roda Husman, 2005). The virus is highly prevalent in surface water and was found in concentrations between 5 and 5×10^3 detectable PCR units per liter of water in rivers in the Netherlands (Lodder and de Roda Husman, 2005). In a study of Borchardt et al. (Borchardt et al., 2003) household wells located near septage land application sites or in rural subdivisions served by septic systems in Wisconsin were tested for the presence of human pathogenic viruses. One out of 50 wells was found positive for human norovirus GII, indicating that even if well water is used, noroviruses

might be introduced into fresh produce chains via irrigation water. The concentration of NoV in the water was not reported.

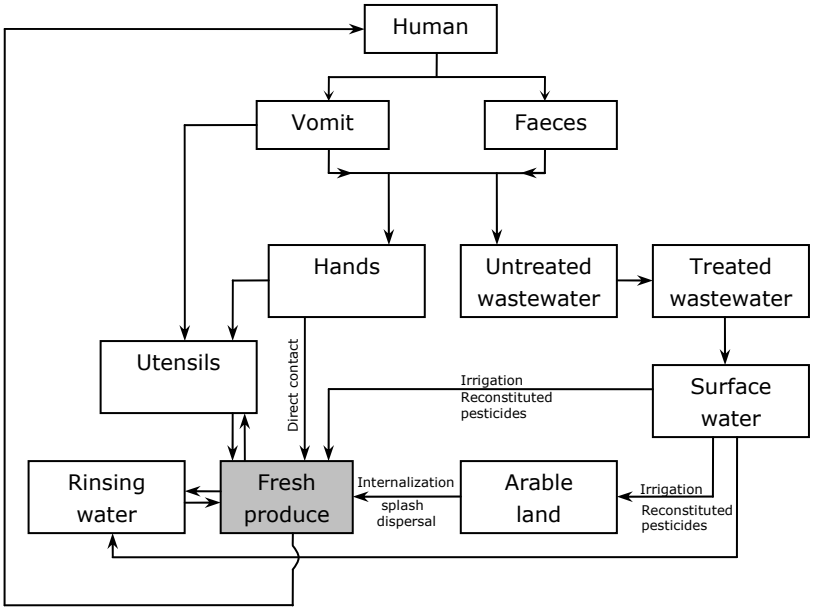

Figure 5 Conceptual exposure pathway with the most predominant routes for NoV-contaminated fresh produce.

In the European project VITAL 91 water irrigation water samples collected in four European countries were examined for the presence of norovirus (Maunula et al., in prep.; Kokkinos et al., 2012). Of these 91 samples, three were positive for NoV. The average NoV-concentration over all samples was estimated as part of the current QVRP at 0.3 PDU per L water (95% interval: 0.15 – 0.50). The corresponding Gamma-distribution mounts to Gamma(0.00004, 6634).

4.3.2 Volume of irrigation water retained by fresh produce

The volume of irrigation water that is retained by produce firstly depends on the type of irrigation that is applied. With spray irrigation, water is aerated across the arable land. With drip irrigation, a system of hoses is distributed across the field to drop water close to the roots of trees, branches or bushes. During spray irrigation, the probability that water contaminates the produce is likely to be greater than with the more localized drip irrigation. This QVRP will therefore consider spray irrigation at present.

The net contamination level due to irrigation depends on the volume of water in contact with the produce, the concentration of viruses in the irrigation water, and the probability that viruses attach to the produce. Spray irrigation schemes are developed to provide each plant with a certain volume of water. It is therefore reasonable to assume a random distribution of the water across the crop field during irrigation. Under this assumption, the volume of water that reaches a lettuce head can be estimated from the amount of water sprayed across the field (either a combination of flow rate and time applied, or total volume specified) and the surface area of a lettuce head. Assuming a lettuce

head is half of a round sphere with radius *r* at time *t*, then the surface area can be approximated by $2\pi r^2$. Combining the volume of water per unit surface area, the surface area of the lettuce head and the concentration of viruses per L irrigation water gives the estimated number of viruses that are deposited on a lettuce head for an irrigation event at time *t*.

An alternative approach that has been used in QMRA is based on measurements by Shuval et al. (1997). The authors assessed the total carrying capacity of water for cucumber and long leaf lettuce by full immersion of the produce in water. The average weight increase after immersion was taken as a measure for the volume of water clinging to the produce. For cucumber, this volume was estimated at 0.36 ml per 100 g, for long leaf lettuce at 10.8 ml per 100 g. These figures might be considered as worst case situation for the volume of water that can be retained by a type of produce. Hamilton et al. (2006) collected produce from fields that were spray irrigated and measured the volume of water retained. For broccoli, the average water retention was estimated at 1.9 ml per 100 g, for Savoy King/Grand Slam cabbage at 3.5 ml per 100 g and for Winter Head cabbage at 8.9 ml per 100 g. Hamilton and colleagues (2006) provided parameters of a log-logistic distribution (α=4.264, β=1.583 10^{-2}, λ=1.085 10^{-3}) for broccoli and empirical CDFs for the cabbages.

Data about the probability of human pathogenic viruses attaching to the lettuce head are lacking. Petterson (2002) has estimated this probability for a bacteriophage to be about 2%, and supplied a beta-distribution with an α of 0.82 and a β of 33.91 to describe the variation in this fraction. An alternative approach might be considered to examine a worst case situation, by considering the attachment of all viruses that reach the produce through the irrigation water.

Petterson et al. (2002) showed experimentally that the last irrigation event before harvest is the determinant for the number of *Bacteroides fragilis* on lettuce heads. This finding may validate the simulation of a single irrigation event in a QVRA that included the irrigation process rather than multiple irrigation events.

4.3.3 Fraction of harvester's hands and food handlers' hands contaminated

In a study on worker's hands in a green pepper bell production chain, 0 of 36 workers had contaminated hands before harvest, whereas five hands (14%) were contaminated after three hours of work (León-Félix et al., 2010). The increase is statistically significant (p=0.03). Green pepper bells collected from the field showed an estimated unit prevalence of 45% (9 out of 20).

In the FP7 European project VITAL, monitoring along the soft fruit and salad vegetable food supply chains was conducted. Harvesters' and food handlers' hands were not found to be contaminated in the soft fruit production chain (number of hands examined: 114), whereas for the lettuce production chain a single harvesters' hand was found to be contaminated (with NoV GG2) out of 101 hands examined (Kokkinos et al., 2012). The estimated number of NoV for the total hand was 84 PDU (95% interval: 20-234).

The proportion of harvesters' hands being contaminated with NoV can be modelled with a beta distribution as Beta(1, 115) (Vose, 2000). This approach makes use of so-called prior information on the modelled proportion, which in the case of the Beta(1, 115) distribution equals a uniform distribution between 0

and 1. This distribution can be viewed as non-informative, because each probability has an equal likelihood of occurring. An alternative prior distribution could be Beta(0.15, 4), which indicates with 95% likelihood a proportion <25%. The resulting Beta distribution to use is then Beta(0.15, 118).
In addition to human pathogenic viruses, human adenovirus (HAdV) was monitored as index virus for faecal pollution. HAdV was detected on 35 (15%) of 239 hand swabs. Following the approach as described for NoV on hands the proportion of faecally polluted hands can be modelled by a Beta(36, 205). This distribution describes an average proportion of 15% and a 95% interval from 11% to 20%. A correlation between HAdV detection and NoV detection on hands was not present in these data, and therefore the conditional probability of NoV contamination given HAdV contamination cannot be assessed.

4.3.4 Virus transfer rates

Virus transfer from hands to a product can occur at several stages of production: during harvest, during food handling at processing, at retail in stores or at markets by personnel or customers, and at preparation by caterers or household members. The amount of viruses that transfer from hands to the product depends on the number of viruses per specified surface area of the hand, the surface area of the hand that touches the product and the rate at which viruses transfer from hands to product.

The surface area of a hand was estimated to average 0.084 m^2 for males and 0.075 m^2 for females (USEPA, 1997). Lettuce heads can be assumed to be picked using a whole hand and therefore the one-sided hand surface of ~0.04 m^2 can be used for risk estimation. Verhaelen et al. (2013a) estimated the surface of a finger pad touching a raspberry using the thumb, index finger and middle finger by staining the finger tip with ink, pressing it onto a paper of known surface area, digitizing the image and using an algorithm to estimate the surface area of the finger tip. No statistical difference was found between these three finger types, suggesting the data per finger can be pooled. The estimated average surface area for a finger tip touching a raspberry was 0.70 cm^2. For three fingers, the total contact area between fingers and raspberries was therefore 2.1 cm^2.

The transfer of norovirus, or its surrogates, has been examined in several experimental studies for several materials. The estimated transfer percentages, and 95% confidence interval, when available, are shown in Table 10.

For food handlers' hands the same modelling approach as for virus transfer from harvesters' hands can be used, with the addition that part of the previous contamination of the product can be removed by the food handlers' hands. This process can be modelled by accounting for the surface touched by the food handler's hand and virus-specific transfer rates from product to hands.

4.3.5 Norovirus concentration in rinsing water

Rinsing water can be tap water, well water or surface water. In the case of tap water, the introduction of viruses to a batch is likely to be sufficiently low to support neglecting it. In case of well water, the vulnerability of the well to microbiological contamination and the depth of the well are important determinants for the microbiological quality of the water (Schijven et al., 2010). For human pathogenic noroviruses, any contamination of well water most likely occurs due to leakage from septic tanks and sewers. The distance between the leakage and the well is then an important determinant for the amount of viruses

that reach the well water. Especially the attachment and detachment rates to and from e.g. sand grains affect the concentrations of viruses in the water, with longer transport distances lowering the amount of passaged viruses (Schijven et al., 2010).

Table 10 Estimated transfer fractions and 95% confidence interval (CI) from or to fresh produce for norovirus or its surrogates

				Time of		% transfer	
Virus*	**Measure**	**Donor**	**Recipient**	**drying†**	**contact**	**(95% CI)**	**Reference**
FCV	Infectivity	Finger	Lettuce	20 min	10 s	18 (7–29)	(Bidawid et al., 2004)
FCV	Infectivity	Lettuce	Finger	20 min	10 s	14 (7–21)	(Bidawid et al., 2004)
FCV	Infectivity	Steel	Lettuce (dry)	0 min	15 s	7 (NR)	(D'Souza et al., 2006)
FCV	Infectivity	Steel	Lettuce (dry)	60 min	15 s	4 (NR)	(D'Souza et al., 2006)
FCV	Infectivity	Steel	Lettuce (wet)	0 min	15 s	5 (NR)	(D'Souza et al., 2006)
FCV	Infectivity	Steel	Lettuce (wet)	60 min	15 s	0.2 (NR)	(D'Souza et al., 2006)
MNV	Infectivity	Glove	Lettuce	2 h	5 s	9 (4-15)**	(Verhaelen et al., 2013a)
MNV	Infectivity	Lettuce	Glove	2 h	5 s	5 (3–8)**	(Verhaelen et al., 2013a)
MNV	Genomes	Glove	Lettuce	2 h	5 s	19 (12-29)**	(Verhaelen et al., 2013a)
MNV	Genomes	Lettuce	Glove	2 h	5 s	22 (16-30)**	(Verhaelen et al., 2013a)
MNV	Infectivity	Glove	Raspberry	2 h	5 s	1 (0.3–10)**	(Verhaelen et al., 2013a)
MNV	Infectivity	Raspberry	Glove	2 h	5 s	6 (<0.1–12)**	(Verhaelen et al., 2013a)
MNV	Genomes	Glove	Raspberry	2 h	5 s	0.1 (0.02-0.2)**	(Verhaelen et al., 2013a)
MNV	Genomes	Raspberry	Glove	2 h	5 s	11 (6-16)**	(Verhaelen et al., 2013a)
NoV1	Genomes	Glove	Lettuce	2 h	5 s	4 (3-7)**	(Verhaelen et al., 2013a)
NoV1	Genomes	Lettuce	Glove	2 h	5 s	23 (18-30)**	(Verhaelen et al., 2013a)
NoV1	Genomes	Glove	Raspberry	2 h	5 s	0.2 (0.06-0.5)**	(Verhaelen et al., 2013a)
NoV1	Genomes	Raspberry	Glove	2 h	5 s	16 (9-24)**	(Verhaelen et al., 2013a)
NoV2	Genomes	Glove	Lettuce	2 h	5 s	6 (3-10)**	(Verhaelen et al., 2013a)
NoV2	Genomes	Lettuce	Glove	2 h	5 s	27 (17-39)**	(Verhaelen et al., 2013a)
NoV2	Genomes	Glove	Raspberry	2 h	5 s	0.1 (0.02-1)**	(Verhaelen et al., 2013a)
NoV2	Genomes	Raspberry	Glove	2 h	5 s	17 (7-27)**	(Verhaelen et al., 2013a)

* FCV: feline calicivirus; MNV: murine norovirus; NoV1: norovirus genogroup 1; NoV2: norovirus genogroup 2; † of donors; ‡ NR: not reported; ** preliminary results

When surface water is used for rinsing, the same approach to the modelling can be used as was described for irrigation water consisting of surface water.

4.3.6 Removal rate of norovirus from product due to rinsing

Depending on the quality of the water used for rinsing, this process can contribute to the virus contamination by addition and removal of virus. The addition can result from viruses already present in the rinsing water before start of washing, or due to contamination of the water by already-contaminated lettuce heads. The rinsing water samples that have been collected in VITAL were found not to contain viruses. The addition of viruses due to rinsing was therefore assumed to be negligible in the current study.

The removal of viruses due to rinsing has been subject of several experimental studies (Barker et al., 2004; Bidawid et al., 2004; Kampf et al., 2005; Kramer et al., 2006; Macinga et al., 2008). These data have been analyzed jointly by Mohktari and Jaykus (2009) and the effect of rinsing with clean water was best described by a uniform distribution indicating virus removal to be between 1 and 2 $\log_{10}$ units. In absence of new data, this removal efficiency can be used in QVRA.

4.3.7 Persistence of norovirus on fresh produce

Because no robust cell culture system for the detection of infectious human noroviruses is available (Duizer et al., 2004), information on the persistence of infectious virus particles in the environment is limited. Due to the non-enveloped structure of noroviruses, which is similar to those of other human enteric viruses, such as polio-, Coxsackie- and echovirus, noroviruses are presumed to be as resistant to environmental degradation and chemical inactivation as the other culturable human enteric viruses. Whether this stability is indeed comparable has to be studied, in the absence of an infectivity assay, by viral surrogates for human norovirus. At present, the most promising surrogate is the culturable murine norovirus due to its genetic similarity and environmental stability (Bae and Schwab, 2008). In general, infectivity reduction rates of surrogates were shown to be higher at higher temperatures (> 25 ºC) and room temperature than at 4ºC as was studied for matrices such as surfaces of stainless steel, lettuce, berries, deli ham, surface and ground waters (Bae and Schwab, 2008; Butot et al., 2008; Cannon et al., 2006; D'Souza et al., 2006). Also, the relative humidity is an important determinant for survival in the environment (Cannon et al., 2006; Stine et al., 2005). Data obtained by the stability of norovirus-like particles as well as surrogate viruses demonstrated stability over a pH range of 3 – 7 and up to 55ºC (Ausar et al., 2006; Cannon et al., 2006; Duizer et al., 2004).

Viruses on foods are challenged by the applied or natural production conditions, such as storage temperature, storage humidity and exposure to sunlight (Table 11). Furthermore, the food matrix itself can induce virus inactivation due to e.g. effects of pH or the presence of proteases. For most intact fresh produce no recommended storage temperature is given in legislation. In general, low temperatures and high relative humidity are applied in the fresh produce industry, to prolong the shelf life and to maintain produce quality. Unlike for bacteria these conditions generally promote viral persistence. The usual storage temperature of lettuce is about 4 °C with a relative humidity of about 80 %. The shelf life of lettuce is strongly dependent on storage conditions. A shelf-life of 21 to 28 days can be expected at 0 °C with >95% RH; at 5 °C a shelf-life of 14 days can be expected. At point of sale, whole lettuces are usually stored at ambient temperature. Persistence of feline calicivirus (FCV) on lettuce at commonly applied storage conditions was studied by Mattison et al. (2007). At 21 °C the virus was not detectable afterfour4 days of storage, which was equivalent to a reduction of about 2.5 $\log_{10}$-units. Infectious FCV was reduced about 2 $\log_{10}$-units after seven days of storage at 4 °C. Murine norovirus (MNV-1) was found to be persistent on raspberries and strawberries at 4 and 10 °C, meaning that the D-values (first 1 $\log_{10}$-unit reduction) exceeded or reached the shelf life of the berries of 7 days.

Table 11 Studies on the natural persistence of foodborne viruses and their surrogates

Virus	Matrix	Conditions	Reference
MNV-1, FCV	Buffer, stainless steel	1. pH 2-10 2. 56 °C, 63 °C, 72 °C 3. organic solvents: Freon, chloroform, vertrel 4. 4 °C stainless steel (7d, wet and dry)	(Cannon et al., 2006)
FCV, CaCV	Buffer	pH 2, 37 °C, 30 min	(Duizer et al., 2004)
NoV GI, NoV RNA, FCV	Stainless steel, formica, ceramic	1. Room temperature, 7d	(D'Souza et al., 2006)
FCV	Lettuce, strawberry, ham, stainless steel	1. 4 °C, 7d 2. Room temperature, 7d	(Mattison et al., 2007)
NoV GI/II, FCV, HAV, RV	Blueberry, raspberry, strawberry, basil, parsley	20 °C for 2, 30, 90 days	(Butot et al., 2008)
MNV-1	Spinach, onions	21 °C, 6 months	(Baert et al., 2008)
MS2	Strawberry lettuce, tomato, parsley and more	4, 8, 22 °C, 7 days	(Dawson et al., 2005)
FCV	Medium, cover slip (dried state)	1. 4, 20, 37 °C suspension 2. 4, 20, 37 °C dried state	(Doultree et al., 1999)
FCV, E-coli, MS2	Lettuce, cabbage	4 °C, 25 °C, 37 °C for 21 days	(Allwood et al., 2004)
E-coli, Shigella, Salmonella enterica, Clostridium perfringens, HAV, FCV, PRDI	Cantaloupe, Lettuce, Bell Peppers	Light exposure, humidity, 22-24 °C, 14 days	(Stine et al., 2005)
HAV	Stainless Steel	1. Humidity (25%, 55%, 80%), 2. Temperature (5 °C, 20 °C, 35 °C)	(Mbithi et al., 1991)
Poliovirus	Lettuce, green onion, cabbage, raspberries, frozen strawberries	4 °C for 15 days	(Kurdziel et al., 2001)
NoV GI/II, mNoV, HAdV	Gloves, raspberries, strawberries, lettuce	4°C, 10°C, 21°C for 1, 3 and 7 days	(Verhaelen et al., 2012)

However, MNV-1 infectivity dropped about 1.5 $\log_{10}$-unit on strawberries after just one day of storage at room temperature, whereas no virus decay was observed on raspberries in this period. Only after three days a 1 $\log_{10}$-unit decrease in MNV-1 infectivity occurred on raspberries at room temperature. Yet, in practice raspberries are rarely stored over such a long period due to the perishability of the product. Assuming a similar persistence for MNV-1 and hNoV, hNoV is therefore likely to stay infectious on raspberries during retail at all tested conditions (Verhaelen et al., 2012). The latter paper also provides a

general model that can be used to estimate a temperature-dependent inactivation rate for a monophasic exponential decay model. The average daily reduction (ADR) for norovirus on soft fruits based on RT-PCR detection was modeled as $10^{-\mu+\beta\times T}$. The value for μ was estimated at 2.36 (95% interval: 2.0 – 2.9), the value for β at 0.077 per day (95% interval: 0.055 – 0.103) (Verhaelen et al., 2012). The *D*-value can subsequently be estimated as 1/ADR. For example, the mean ADR for norovirus at 4°C is $10^{-2.36+4\times0.077}$=0.01 and the corresponding *D*-value amounts to 113 days.

4.3.8 Consumption of fresh produce

To date, very limited data are available for the consumption of fresh produce. EFSA's comprehensive food consumption database specifies consumption data on aggregated levels (e.g., total consumption of vegetables per day, including mushrooms and other fungi) (EFSA, 2011b). Thus, specific data for a particular product are not retractable from the database. Table 12 displays the consumption data for the EFSA database for salad vegetables and soft fruits.

As reported for consumption of shellfish and pork meat, a national food consumption survey was recently conducted in the Netherlands, where people between 6 and 79 years of age were asked to register food intake during two days. Data for fresh produce consumption are not yet available when this report was finished, but are expected to become available in 2013.

4.3.9 Dose response model

Teunis et al. (2008) described a dose response relation to estimate the probability of infection and the conditional probability of illness given infection. These are the only quantitative models available for QVRA of norovirus to date. The models are based on experiments with volunteers ingesting an inoculum with an predetermined number of *norwalk* virus particles (*norwalk* virus is a particular strain of norovirus, belonging to genogroup I). The probability of infection for a given NoV dose D was calculated as:

$$P_{inf}(D \mid \alpha, \beta) = 1 - {}_1F_1(\alpha, \alpha + \beta \mid -D) \qquad (7)$$

where parameters α and β characterize the infectivity and ${}_1F_1$ represents a confluent hypergeometric function. Values for α and β are available as Monte Carlo set, from which a combination can be drawn randomly for each of the doses estimated in the Monte Carlo simulation of the QVRA. This dose-response model thereby introduces heterogeneity in susceptibility for norwalk virus, as was observed among the volunteers. By applying this approach in the QVRA for norovirus means that the heterogeneity for the experimental population is the same as that for the population of fresh produce consumers. Data lack at present to conclude on the validity of that assumption.

Another point of attention in using this dose response model is that the particular strain of norovirus used, i.e., norwalk virus, is considered highly infectious, where this infectivity remains to be assessed for other variants of norovirus. In this perspective, however, the use of this dose response model could be interpreted as a worst case approach, and any intervention measure that is anticipated to reduce public health risks sufficiently might be similarly or more effective in practice for non-norwalk noroviruses.

Table 12 Food consumption data for soft fruits and salad vegetables in The Netherlands, abstracted from the EFSA comprehensive food consumption database (EFSA, 2011b)

Age category	N	Mean	Std	P5	P10	Median	P95	P97.5	P99
Fruit and fruit products									
Adults	750	94.8	110.7	0.0	0.0	64.2	330.0	382.0	477.1
Toddlers	322	111.3	75.8	0.0	6.7	107.1	234.7	270.0	366.3
Other children	957	110.3	80.8	0.0	0.0	102.4	259.3	294.2	349.4
Vegetables and vegetable products (including fungi)									
Adults	750	105.1	78.3	0.0	15.0	90.6	249.7	303.1	352.1
Toddlers	322	38.5	35.0	0.0	0.0	30.5	104.0	136.4	169.0
Other children	957	39.6	38.2	0.0	0.0	31.2	111.1	149.2	174.7

N: number of persons in the study; Mean: mean g ingested per day

The dose response relation for the probability of illness conditional on infection was calculated using equation (8):

$$P_{ill}(D \mid \eta, r) = 1 - (1 + \eta D) - r \qquad (8)$$

with $\eta=2.55\times10^{-3}$ and $r=0.086$ (Teunis et al., 2008).

5 General discussion and conclusions

5.1 General discussion

The majority of published studies on the contamination of products with enteric viruses report percentages of samples detected positive (so-called prevalence studies). Such prevalence studies are mostly as good as the method used and therefore yield method-dependent data. Factors that affect the data quality include the limit of the detection assay, the inefficiency in amplification, the RNA isolation efficiency, the procedure used for quantification and whether or not shellfish are pooled prior to nucleic acid isolation. Approaches have been published to account for several of these aspects in de detection protocol (Costafreda et al., 2006; D'Agostino et al., 2011; Lees, 2010). Nevertheless, when proper controls have been included in the analyses, data from published papers were found to be lacking sufficient detail for proper inclusion of the results in risk assessment studies. A solution to this problem is to obtain the raw data from the original scientists. When made available, then virus concentrations including their uncertainty and variation can be estimated and included in the risk estimation.

Another general shortcoming for QVRA is the lack of methods available for absolute quantification of viruses. When cell culture systems are available, the number of cytopathological effects in cell culture, or plaques in a plaque assay, can be interpreted as indicator for number of viable viruses per unit of sample examined. When such systems are not routinely available, as is the case for NoV, HAV and HEV, however, one is restricted to the use of indirect methods such as genome detection by (RT-)PCR. The methods for correct use of qPCR data in QMRA are lacking, which leads to the unavoidable–at present–ignorance of two aspects: 1) distinction between genomes from infectious (viable) and non-infectious (non-viable) microorganisms, and 2) uncertainty and bias in absolute quantification of genomes. Distinction between infectious and non-infectious microorganisms cannot be made with qPCR directly, because the detected genomes originate from both types of microorganisms. Approaches are being examined at present to distinguish the two by using for instance preenzymatic treatment (Schielke et al., 2011). Furthermore, results generated by qPCR are, when quantified, translated into point-estimates of genome quantities according to a standard curve based on samples with supposedly known concentrations of targets (i.e., the standards). Often neglected in such quantification are the efficiency of isolation of genomic material from samples, differences in amplification efficiency between the standards and targeted microorganisms, uncertainty around the estimated concentration of the standards and target viruses, and measurement error of the apparatus.
To properly use the results of molecular methods for viruses in quantitative risk assessment, current procedures need to be adapted for adequate quantification – with uncertainty – of infectious microorganisms detected by qPCR.

5.2 Discussion on HAV in shellfish

Estimation of risk assessment parameters for HAV in shellfish largely encompass data for HAV in shellfish culturing waters and shellfish. Moreover, most screening data for viruses in shellfish considered HAV or NoV whereas most experimental data included enteroviruses, mostly poliovirus vaccine strains. With regard to the shellfish most data concerned oysters. These are important

aspects to take into account if a full quantitative risk assessment for HAV in shellfish is the aim, since extrapolation of data for other viruses or shellfish may not be representative in all cases.

Another important aspect regarding prevalence studies is the origin of the shellfish. For several studies, it was not clear where the shellfish came from and how they were produced. The latter is especially important for the development of possible risk management options. In the case of shellfish, it is mostly the microbiological quality of the water that drives the microbiological quality of shellfish. If shellfish are imported into a country and relayed locally for only a short period of time, then the intervention measures should affect the exporting country's growing area and not the local growing area. Thus, the origin of the shellfish examined should be accounted for either in the design of a risk assessment study or in the inclusion of already collected data into a risk assessment.

In terms of management options to improve the microbiological quality of shellfish, the transmission pathway earlier in the trajectory should be considered. This approach involves more data on the different processes that need to be considered, such as virus concentration in surface water, filtration rates of shellfish and the virus-specific retention rates. Initial estimates are provided for the retention rate of HAV in mussels, but the data base is scarce and therefore the estimates are likely not robust. In the ideal situation, modelling a virus' fate and behaviour in the full trajectory from surface water to shellfish consumption could enable the comparison of different intervention measures. Furthermore, it aids to interpret a certain virus concentration in surface water in terms of health risks due to oyster consumption. The latter is important to evaluate possible standard setting and monitoring options, such as environmental surveillance. Environmental surveillance could be attractive as alternative to end product monitoring, because pathogen concentrations in growing waters could provide more general information on the total population of shellfish present compared to actual shellfish monitoring.

Management of marine waters for protecting consumer health has largely focused on the presence and concentration of faecal indicator bacteria both in waters and in shellfish tissue. Bacterial indicators are known to be poor indicators of viral contamination, however as yet, no suitable indicator for viruses has been identified.

5.3 Discussion on HEV in pork

Pork products are diverse in nature and content. The magnitude of the public health risk will depend on the actual pig parts that are included in the final product, the risk of cross-contamination with faeces, bile or blood, and any processing of the pork product. At present, especially limitedly processed, locally produced, sausages have been associated with sporadic HEV infections in humans, e.g. in Southern France (Colson et al., 2010). Given the large portion of subclinical infections in humans that is expected to exist (Bouwknegt et al., 2009a), however, such observed cases may be the severe episodes. An increased response was observed with increasing dose ingested for pigs (Meng et al., 1997). If humans respond similarly, then the raw, limitedly processed sausages may lead to a higher ingested dose than pork products that have been processed more intensively or comprise different pig tissues. Therefore other pork products might also contribute to the total public health burden for HEV, albeit at currently unknown levels. Quantitative virological risk assessment is

then a valuable to estimate the total burden. Important data gaps, however, exist for doing such an assessment, as described in this report.

At present, a cell culture system for HEV is unavailable. The lack of such a system provides additional challenges for quantitative virological risk assessment regarding HEV. Clearly, the earlier described distinction between infectious and non-infectious viruses cannot be made, which may lead to an overestimation of the actual infection risks. Furthermore, studies on the persistence of HEV under exposure to a potential virucidal condition, such as high temperature or low pH, cannot be done. Such virucidal effects are, however, important to consider in risk assessments. On the one hand, neglecting such effects may cause the actual risk to be overestimated and likewise the efforts required to reduce the risk. On the other hand, the effects of potential intervention measures cannot be assessed robustly, hampering the development of efficient public health policy. It would therefore be highly beneficial if a cell culture system would become available for HEV.

Another important data gap for HEV is the lack of a dose-response model for humans. Currently, a single dose-response model is available for pigs, based on intravenous inoculation of pigs, whereas HEV in most likely transmitted faecal-orally (Bouwknegt et al., 2011). A correction factor based on experimental data was used to adjust for the intravenous route of inoculation. Whether this dose response model is representative for humans, however, remains unknown. Pigs have many similarities to humans with regard to body structure and tissue and organ function. More specifically, humans and pigs share similar organ size, feeding patterns, dietary habits, kidney structure and function, pulmonary vascular bed structure, respiratory rates, social behaviours and digestive tract physiology (Tumbleson and Schook, 1996). Especially this similar physiology suggests that intestinal uptake of HEV might be comparably efficient between pigs and humans. Once in circulation HEV needs to infect liver cells (hepatocytes) equally efficient in pigs as it does in humans for the porcine dose response model to be valid for human infection risk prediction. An approach to examine this similarity involves the culture of porcine and human hepatocytes and to test the efficiency of cell invasion on both cell lines. The absence of such a cell culture system, however, and the difficulty in obtaining fresh hepatocytes that can be maintained in culture hampers such experiments. Alternatively, a comparison of the receptor(s) utilised by HEV to enter porcine and human hepatocytes could provide such information on the infection efficiency comparability between the two species. The receptor(s), however, have not been identified to date, hampering the structural and physiological comparison. Pig livers are considered a proper candidate for human liver replacement for patients suffering from e.g. acute liver failure, amongst others due to similar functionality, physiology and size between porcine and human livers (Ekser et al., 2009). Possibly, the functional and physiological similarity also holds for the receptors located on hepatocytes. If this similarity indeed exists, then the dose response model for pigs may be a valid representative for the dose response model for humans. As indicated, however, experimental work is required when proper methods become available to support this statement.

5.4 Discussion on NoV in fresh produce

A potential contamination point in fresh produce production is irrigation water. The magnitude of contamination depends on the volume of water retained by food crops and the concentration of norovirus therein. Both aspects are expected to be variable. Whether or not, and how long, irrigation is applied is driven by

climatic conditions and differs between rounds. And the norovirus concentration in surface water is expected to be highly variable and episodic (Westrell et al., 2006), leading to episodic contamination with higher norovirus loads. It is therefore important to characterize the variation properly, which requires large sample sizes with samples collected longitudinally.

The infection risk for an ingested norovirus dose can be estimated using a described dose response model based on norwalk virus (Teunis et al., 2008). Interestingly, heterogeneity among hosts for the susceptibility to infection is taken into account with this model, because of the probability of an individual norovirus particle to cause infection was modelled as a beta distribution. Ideally, aspects such as genetic susceptibility and acquired immunity against infection and illness are also taken into account, as volunteer studies have shown that some subjects remain uninfected even after challenge with high doses (Johnson et al., 1990; Matsui and Greenberg, 2000). Human ABH histo-blood group antigens and the secretor phenotype may influence susceptibility to NoV, although it is not clear whether these volunteers remain disease-free because of innate resistance or because of pre-existing immunity (Lindesmith et al., 2003). The individuals that experienced a NoV infection acquire short term immunity (Wyatt et al., 1974). Previous studies have shown that most raw oyster consumers were male, young or middle-aged adults, persons of high socioeconomic status, and persons from specific ethnic groups are more likely to eat raw oysters than others (Altekruse et al., 1999; Shapiro et al., 1998). These considerations may affect the immune response against NoV infections of the consumers and therefore, the final risk estimation. If more data become available with respect to distribution of immunities in the population these could be fed into the risk assessment.

Another important source for virus introduction onto fresh produce results from contact transfer, such as from hands or utensils to product and vice versa. The rate at which viruses are transferred from a donor (e.g., hand, produce) to the recipient (e.g., produce, rinsing water) is found to be specific for a particular material or produce (Bidawid et al., 2004; Verhaelen et al., 2013a). For transfer from gloves and steel materials, the transfer rates have been determined experimentally and are thus available for risk assessment, as described in this report. For other materials, however, the required data are lacking and need to be collected.

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