

SCIENTIFIC OPINION

Scientific Opinion on an update on the present knowledge on the occurrence and control of foodborne viruses¹

EFSA Panel on Biological Hazards (BIOHAZ)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

A review of the biology, epidemiology, diagnosis and public health importance of foodborne viruses was performed. Data needs to support a risk assessment were also identified. In addition possible control options and their anticipated impact to prevent or reduce the number of foodborne viral human infections were identified, including the scientific reasons for and against the establishment of food safety criteria and process hygiene criteria for viruses for certain food categories. Food may be contaminated by virus during all stages of the food supply chain, and transmission can occur by consumption of food contaminated during the production process (primary production, or during further processing), or contaminated by infected food handlers. Transmission of zoonotic viruses (e.g. HEV) can also occur by consumption of products of animal origin. Viruses do not multiply in foods, but may persist for extended periods of time as infectious particles in the environment, or in foods. At the EU-level it is unknown how much viral disease can be attributed to foodborne spread. The relative contribution of different sources (shellfish, fresh produce, food handler including asymptomatic shedders, food handling environment) to foodborne illness has not been determined. The Panel recommends focusing controls on preventive measures to avoid viral contamination rather than trying to remove/inactivate these viruses from food. Also, it is recommended to introduce a microbiological criteria for viruses in bivalve molluscs, unless they are labelled “to be cooked before consumption”. The criteria could be used by food business operators to validate their control options. Furthermore, it is recommended to refine the regulatory standards and monitoring approaches in order to improve public health protection. Introduction of virus microbiological criteria for classification of bivalve molluscs production areas should be considered. A virus monitoring programme for compliance with these criteria should be risk based according to the findings of a sanitary survey.

KEY WORDS

Food borne viruses, Norovirus, Hepatitis, Microbiological criteria, molluscs, fresh produce.

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² Panel members: Olivier Andreoletti, Herbert Budka, Sava Buncic, John D Collins, John Griffin, Tine Hald, Arie Hendric Havelaar, James Hope, Günter Klein, Kostas Koutsoumanis, James McLauchlin, Winy Messens, Christine Müller-Graf, Christophe, Nguyen-The, Birgit Noerrung, Luisa Peixe, Miguel Prieto Maradona, Antonia Ricci, John Sofos, John Threlfall, Ivar Vågsholm and Emmanuel Vanopdenbosch. Correspondence: biohaz@efsa.europa.eu

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Biological Hazards to initiate a self-tasking issue with the purpose to provide up-to-date information on the present knowledge on the occurrence and control of foodborne viruses. The BIOHAZ Panel carried out a review of the available information in the scientific literature with regards to the biology, epidemiology, diagnosis and public health importance of foodborne viruses. Where possible the review covered primary production, food harvesting, food processing, and storage/retail until consumption. Data needs to support a risk assessment were also identified. In addition possible control options and their anticipated impact to prevent or reduce the number of foodborne viral human infections were identified including the scientific reasons for and against the establishment of microbiological criteria for viruses for certain food categories (e.g. fresh produce, bivalve molluscs etc).

The opinion draws conclusions on the biology, epidemiology, diagnosis and public health importance of the foodborne viruses Norovirus (NoV), Hepatitis A virus (HAV) and Hepatitis E virus (HEV).

NoV infection is the most common cause of infectious human gastro-enteritis. NoV is shed in huge quantities in the stool and vomit of infected persons, and oral exposure to only a few particles is sufficient to cause disease. HAV is the aetiological agent of the most common type of hepatitis worldwide. Infectivity is unknown but may be very high. In contrast to NoV and HAV, HEV has been identified also as a zoonosis. Although rare, its importance is increasingly recognised in the EU. The dose response relationship for HEV for humans, is unknown.

In the EU, the major mode of transmission for NoV remains person-to-person (directly from the human reservoir). In the EU, the major mode of transmission for HAV is directly or indirectly from the human reservoir, mainly as a consequence of travelling to endemic regions, having risky sexual practices or consuming contaminated water or food.

Food may be contaminated by virus during all stages of the food supply chain, and transmission can occur by consumption of food contaminated during the production process (primary production, or during further processing), or contaminated by infected food handlers. Transmission of zoonotic viruses (e.g. HEV) can also occur by consumption of products of animal origin, although few cases are reported. Viruses do not multiply in foods, but may persist for extended periods of time as infectious particles in the environment, or in foods.

At the EU-level it is unknown how much disease caused by NoV can be attributed to foodborne spread. Studies in some countries suggest that this can be significant. The relative contribution of different sources (shellfish, fresh produce, food handler including asymptomatic shedders, food handling environment) to foodborne illness has not been determined. Current EU surveillance for foodborne NoV illness does not capture dispersed outbreaks very efficiently, and there is clear evidence of significant underreporting of foodborne NoV outbreaks. The background data from case reports of HAV is often insufficient to prove foodborne transmission, but occasional outbreaks have been documented. With the decreasing immunity to HAV in the EU population, the probability of outbreaks is increasing. The diagnosis of HEV infections in humans is not routinely done in most laboratories, and therefore, there is considerable under diagnosis of this infection and illness.

Possible control options and their anticipated impact to prevent or reduce the number of foodborne viral human infections are given in the opinion together with several recommendations.

Thus, it is recommended to focus on preventive measures to avoid viral contamination rather than trying to remove/inactivate these viruses from food. Also it is recommended to introduce microbiological criteria for viruses in bivalve molluscs, unless they are labelled “to be cooked before consumption”. These criteria could be used by Food business operators to validate their control options to meet the established virus criteria. Using an *E. coli* standard for monitoring and classification of bivalve mollusc production areas provides general information about the background level of faecal contamination, and is recommended to be retained.

Furthermore the regulatory standards and monitoring approaches could be refined to improve public health protection. Introduction of virus microbiological criteria for classification of high risk bivalve molluscs (to be consumed raw) production areas should be considered. A virus monitoring programme for compliance with these criteria should be risk based according to the findings of a sanitary survey.

It is also recommended that EU environmental legislation considers specific protection against faecal pollution to bivalve mollusc production areas. Control measures need to focus on avoiding faecal contamination in mollusc production areas as much as possible. Sanitary surveys would provide the necessary knowledge base. Preventative approaches could include: introduction of prohibition zones in the proximity of sewage discharges, more stringent *E. coli* standards for class B classification areas, and the use of pollution alert procedures.

Post-harvest treatments need to be validated for virucidal activity (e.g. using HAV as a model) to ensure that the treatments are effective, and can be applied consistently prior to implementation in the food production chain. In addition further training of food handlers about hygiene requirements and about specific viral contamination of foods and food preparation environment is recommended in order to reduce the risk of contamination of ready-to eat foods. Finally it is recommended that high risk groups (people with underlying liver disease, immuno-compromised persons and pregnant women) should be discouraged from eating meat and liver derived from wild boars and domestic pigs without proper cooking for prevention of hepatitis E.

In the opinion data needs to support a risk assessment have also been identified. Thus routine harmonised surveillance of NoV, and of virus occurrence in food commodities including molecular typing is recommended to aid source attribution studies. For HEV and HAV, notification and systematic strain typing of viruses in humans and in animals (HEV) and food commodities (HAV) are needed to get a better understanding of sources of virus. Studies are also needed to determine the importance of foodborne transmission pathways for HEV.

To determine the burden of disease, including foodborne illness, population-level estimates of incidence, risk factors, and clinical impact of NoV, HAV, and HEV in humans in general, and in specific risk groups (e.g. immuno-compromised individuals, elderly) are needed. Studies are also needed to determine the importance of presymptomatic, postsymptomatic, and asymptomatic shedding of NoV and HAV as sources of foodborne human infection.

In order to quantify the efficacy of specific control options, it is necessary to build a quantitative risk assessment framework. This should be done for specific priority virus-commodity combinations, including consideration of the target population. Data needs for QMRA of FBV include: consumer habits, virus contamination levels in food and other reservoirs, virus transfer rates, natural persistence on/in foods (at the pre-harvest and post-harvest levels), and human dose-response relations. These data should be collected based on specific targeted studies, including sampling strategies. In addition, more studies are needed on the relation between detection of virus genomic copies by PCR in food and probability of causing disease. For this purpose, a guidance for outbreak investigation for FBV-related outbreaks could be drawn up to generate the type of data needed for QMRA.

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BACKGROUND AS PROVIDED BY EFSA

In the EU, viral agents were responsible for 11.9% of the foodborne outbreaks reported to the European Food Safety Authority (EFSA) during 2007⁴ and were identified as the second most common causative agent group, after Salmonella. Also, Member States identified foodborne viruses as a relevant hazard in food at a recent EFSA Network meeting on Microbiological Risk Assessment⁵.

An increased number of foodborne viral outbreaks are recorded in several countries. Reasons for this include the improved diagnostic methods that have enhanced detection of some virus groups, and the increased marketing of fresh and frozen foods that has led to a worldwide availability of high risk food.

Unlike bacteria, viruses do not multiply or produce toxins in food; food items merely act as vehicles for their transfer. Viruses such as hepatitis A virus (HAV), noroviruses, enteroviruses, astroviruses, adenoviruses, rotaviruses and hepatitis E virus have all been implicated in food- and/or water-borne outbreaks of illness. There is a potential for any enteric virus which causes illness when ingested to be transmitted by food, but in practice most reported incidents of viral foodborne illness are due to gastroenteritis viruses and hepatitis A virus.

Numerous foodborne outbreaks caused by viruses have been seen in the EU (EFSA Journal, 2010, 1496). In 2008, 19 MSs reported a total of 697 outbreaks, and for the second year in a row, the total number of outbreaks caused by viruses increased. For those outbreaks that were verified, noroviruses were the most frequent cause, followed by HAV

Apart from tick-borne encephalitis virus, which can be shed by infected dairy animals and subsequently infect humans via milk; and hepatitis E virus which can be transmitted through consumption of undercooked meat, viral foodborne infections are limited to the recycling of human viruses back to humans. Recent studies suggest the presence of noroviruses in pigs and cattle, but there is no evidence for direct zoonotic transmission. It should be emphasized that traditional viral zoonosis such as Rhabdovirus, Hanta virus and Influenza A viruses are **not** considered to be foodborne. Recent outbreaks of avian influenza (AI) have occurred in birds in Europe, in the US in Asia and in Africa. Almost all the reported cases of AI virus infection in humans have been caused by HPAI viruses belonging to the H5 or H7 subtypes and are transmitted directly from infected birds to humans. Other routes of infection, such as consumption of edible tissues from infected avians or contact with contaminated water, have been suggested as possible sources of infection, but have not yet been proven.

Human viruses can contaminate food either through contamination at source, principally through sewage pollution of the environment, or in association with food processing through inadequate hygiene practices of operatives or systems. Consequently many different food items such as vegetables, shellfish and a great variety of ready-to-eat (RTE) foods like sandwiches, cold meat, pastries etc. have been implicated in foodborne viral infections. Bivalve shellfish are commonly involved in outbreaks of foodborne viral diseases. Shellfish are filter feeders and if shellfish-growing waters are polluted with human sewage, the shellfish extract viruses infectious for humans. The difficulties in detecting virus in shellfish pose further problems, as well as the fact that correlation between levels of bacteria indicator organisms and the extent of viral contamination is poor.

The most fundamental problem with regard to detection of virus in foods is that the infectivity is high, for calicivirus approximately 10 particles, and that the viruses of greatest concern, hepatitis A viruses and caliciviruses, can not readily be cultured. New viral test methods based on PCR have been developed but data on the correlation between the presence of viral genes (as tested by PCR) and

⁴ The Community Summary Report on Foodborne Outbreaks in The European Union in 2007.

http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902515341.htm

⁵ Minutes of the 3rd meeting of the EFSA Network on Microbiological Risk Assessment

viable virus are lacking. For outbreak diagnosis, the current approach is the screening of stool samples from cases and controls, combined with an epidemiologic investigation to assess food-specific attack rates.

There is no doubt that food and waterborne viral infections will become an increased challenge to public health in the future. At the same time it will be a great challenge to food microbiologists, virologists and epidemiologists to expand the knowledge on this issue and thereby contribute to the prevention of virus infections through water and food.

Commission Regulation (EC) 2073/2005⁶ lays down food safety criteria. However no specific criteria are set for viruses. The SCVPH issued an opinion on Norwalk-like viruses (NLVs, noroviruses) on 30-31 January 2002. In that opinion it concluded that the conventional faecal indicators are unreliable for demonstrating the presence or absence of NLVs and that the reliance on faecal bacterial indicator removal for determining shellfish purification times is unsafe practice. It also recommended using *E. coli* rather than faecal coliforms to indicate faecal contamination in shellfish harvesting areas, when applying bacterial indicators. The regulation only indicates that criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently.

Regulation (EC) No 853/2004⁷ provides a possibility to lay down additional health standards for live bivalve molluscs in cooperation with the relevant Community Reference Laboratory, including: virus testing procedures and virological standards.

TERMS OF REFERENCE AS PROVIDED BY EFSA

The Biohaz Panel has decided to initiate a self tasking issue with the purpose to provide up-to-date information on the present knowledge on the occurrence and control of food- borne viruses. EFSA requests the BIOHAZ Panel:

1. To carry out a review of the available information in the scientific literature with regards to the biology, epidemiology, diagnosis and public health importance of foodborne viruses. Where possible the review will cover primary production, food harvesting, food processing, and storage/retail until consumption. Data needs to support a risk assessment will also be identified.
2. To identify possible control options and their anticipated impact to prevent or reduce the number of foodborne viral human infections.
3. To discuss the scientific reasons for and against the establishment of food safety criteria and process hygiene criteria for viruses for certain food categories (e.g. fresh produce, bivalve molluscs etc.)

⁶ OJ L 338, 22.12.2005, p. 11,12. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs amended by Regulation (EC) No 1441/2007 (OJ L 322, 7.12.2007, p.17,18)
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005R2073:en:NOT>

⁷ OJ L 139, 30.4.2004, p. 30, 68, Corrigendum to Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April laying down specific hygiene rules for food of animal origin

ASSESSMENT

1. Introduction

Currently known viruses that can infect humans are grouped into 22 families. In addition to this, the recent advances in molecular techniques that allow characterisation of all genetic material in a given sample has led to the identification of several new viruses in recent years, most of which remain to be fully characterised (Allander et al., 2005; Briese et al., 2009; Jones et al., 2007). Foodborne transmission has been documented for viruses belonging to at least 10 of these, and the diseases associated with these infections range from mild diarrhoeal illness to severe encephalitis. Foodborne transmission can occur by contamination of food by infected food handlers, by contamination of food during the production process (e.g. in shellfish production), or more seldom by consumption of products of animal origin harbouring a zoonotic virus.

Table 1: Types of foodborne transmission, and examples of viruses involved.

	Source of contamination		
	Primary production of products of animal origin (virus originating from animal reservoir)	Primary production of fresh produce and/or shellfish (virus originating from human reservoir)	Food handler (virus originating from human reservoir)
Mode of transmission	Meat, blood, milk, saliva	Sewage, irrigation water	Hands, environment, faecal-oral
Foodborne disease	Rare	Frequent	Frequent
Examples	SARS Coronavirus Hepatitis E virus Tick-borne encephalitis virus Nipah virus	Norovirus Hepatitis A virus Hepatitis E virus	Norovirus Hepatitis A virus Hepatitis E virus

While foodborne transmission is possible for multiple viruses, the burden of foodborne illness is thought to be greatest for human viruses that are transmitted through poor hygienic practices, either by food handlers or during food production (Mead et al., 1999). This applies to viruses that are transmitted by the faecal-oral route, hence infect their host after ingestion, followed by invasion of cells in the epithelial lining of the gut, and subsequent replication in the same site or elsewhere in the body. An expert meeting convened under the auspices of WHO/FAO and OIE⁸ reviewed available evidence and grouped viruses according to their ability to cause high morbidity, severe disease, or a significant ability to cause outbreaks. In the WHO/FAO document, the common pathogens noroviruses (NoV), group A rotaviruses, and hepatitis A viruses (HAV) were ranked as priority hazards. In the category of emerging hazards, hepatitis E virus (HEV), Nipah viruses, H5N1 avian influenza viruses and SARS coronavirus were considered to be of greatest concern. Subsequently, available evidence for a specific food-commodity combination was reviewed, by considering available information on estimates of the incidence of foodborne disease linked to a specific commodity, and the level of evidence for the importance of that commodity in causing viral foodborne illness. This resulted in several virus-commodity combinations for which prevention and control measures should be considered:

- *NoV and HAV in bivalve molluscan shellfish*
- *NoV and HAV A in fresh produce*
- *NoV and HAV in prepared foods*
- *Rotaviruses in water for food preparation*
- *Emerging viruses in selected commodities*

⁸ Viruses in Food: Scientific Advice to Support Risk Management Activities. Meeting Report Microbiological Risk Assessment Series, No. 13, 2009; <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=15&codcch=751>

These conclusions were based on available evidence from literature, but it was also noted that large data gaps exist: trends in disease reporting are available in many parts of the world for hepatitis A, but not for the other viruses. Estimates of the proportion of illness caused by these pathogens that can be attributed to consumption of contaminated food are based on very few studies, and would require addition of systematic strain typing to routine surveillance, or more systematic studies to provide the data for burden estimates (similar to the global *Salmonella* surveillance activities). Finally, testing for viruses in commodities is difficult, and there is considerable debate over interpretation of findings, as will be discussed elsewhere in this report. As a consequence, data from product monitoring are patchy at best.

Nevertheless, WHO called for action because evidence for foodborne viral disease is compelling, but this has not yet been translated to the routine practice of food safety authorities. Current EU legislation does provide guidance, for instance by specifying the need for use of high quality water in food production, and stressing food handling hygiene (Appendix C). However, the currently used methods for monitoring and the use of *E. coli* as a microbiological criteria do not correlate consistently with presence or absence of viruses. As a consequence, food industry and food safety authorities at present lack the tools that enable them to monitor virological quality control in contrast with the situation for bacteriological contamination (e.g. *Salmonella*). For shellfish, standardized and validated protocols for virus detection are in final stages of development, but for other commodities this is a distant reality, if at all realistic.

In the present opinion, no systematic assessment of the priority for food borne viruses (FBV) was performed. For the purpose of this opinion, NoV and HAV were covered fully in the food categories proposed in the WHO opinion. In addition, because of the increasing zoonotic concern, HEV is also included, as it is highly prevalent in pigs across Europe, and there is some evidence for foodborne transmission in Europe, although human clinical cases are rare (Lewis et al., 2010).

Since water is outside the scope of this document, rotaviruses will not be covered in this opinion; also they have not been reported to be foodborne to date. Potentially emerging viruses which are uncommonly transmitted by food will be discussed in the hazard identification chapter of this opinion only.

2. Hazard identification

Information about foodborne outbreaks caused by viruses in the EU can be found in The Community Summary Report⁹. In 2008, 19 MSs reported a total of 697 outbreaks, and for the second year in a row, the total number of outbreaks caused by viruses increased. For those outbreaks that were verified, NoV was the most frequent cause, followed by HAV. Crustaceans, shellfish, molluscs and products thereof were the most frequently implicated food items. In addition to this, approximately 27% of the verified NoV outbreaks the implicated foodstuff was unknown. The use of epidemiological criteria in the US concluded that an estimated 28% of all reported outbreaks with unknown aetiology were likely caused by NoV (Turcios et al., 2006). The reporting of outbreaks to EFSA was initiated in 2007, and it is likely that the numbers and proportion of reported viral outbreaks will increase as not all countries are providing data on viral outbreaks, in contrast to *Salmonella* reporting. What the report does not yet provide is insight into the geographic spread of outbreaks. For *Salmonella*, occasional international diffuse outbreaks are identified, caused by widely disseminated products. Identifying such outbreak required systematic incorporation of molecular typing into outbreak investigations and reporting, a practice that is common for *Salmonella* but not for viruses. Indications of this can also be obtained when reviewing notifications from countries about possible food-related incidents in which viruses are involved. This is done through the rapid alert system for food and feed (Data extracted from RASFF, are presented in Appendix 2). The recent increase in RASFF notifications for suspected viral contamination is remarkable, possibly reflecting increasing awareness (Figures 1 and 2). However,

⁹ The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and foodborne outbreaks in the European Union in 2008, *The EFSA Journal*; 2010 8(1):1496.

RASFF notifications are not representative and are not based on common notification criteria. Incident notifications may follow illness reporting, or detection of a virus in a food product, or both. Therefore these figures have to be interpreted with care. At least a tendency for higher awareness of viral agents is visible.

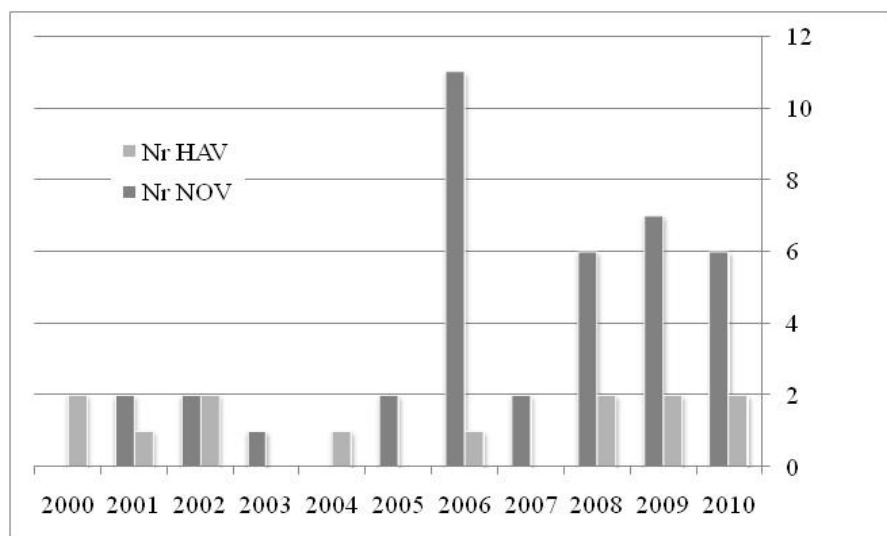


Figure 1: Number of notifications per year for suspected viral contamination of food products through RASFF from 2000 until March 2010.

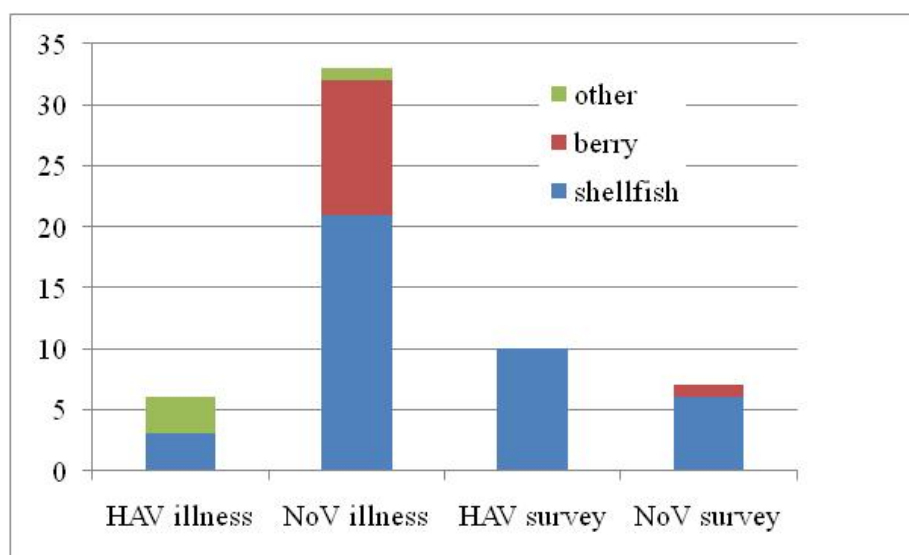


Figure 2: Number of notifications for suspected viral contamination of food products through RASFF from 2000 until March 2010, based on illness reports or virus detection in products

2.1. Norovirus

NoV belong to the Family *Caliciviridae*, that is divided into genera. NoV and *Sapovirus* are the two out of five genera of the family *Caliciviridae* that contain viruses that cause infections in humans. NoV have also been detected in pigs, cattle, mice, cats, dogs, and sheep, and sapoviruses in pigs. The other genera of the family *Caliciviridae* are *Lagovirus*, *Vesivirus*, and *Nebovirus* encompassing viruses infecting rabbits, and brown hares (lagoviruses), sea lions, swine, cats, dogs, fish, seals, other marine animals, cattle and primates (vesiviruses), and cattle (Nebovirus). In humans, NoV and sapoviruses cause gastroenteritis, while the animal viruses can cause a range of different clinical syndromes,

including oral lesions, systemic disease with hemorrhagic syndromes, upper respiratory tract infections and other. Furthermore, one other potential genus comprising viruses detected in rhesus macaques has been described. So far, the NoV and sapoviruses are the only caliciviruses known to cause disease in humans, with the exception of anecdotal zoonotic infection with vesiviruses. NoV can be divided into distinct genogroups, based on phylogenetic analyses of the capsid protein. To date, five NoV genogroups (G) have been recognized (GI-GV). Viruses of GI, GII and GIV are known to infect humans. GII viruses have additionally been detected in pigs, and GIV viruses have been detected in a lion cub and a dog. GIII viruses infect cattle and sheep and GV viruses infect mice. Recombination between viruses from different genogroups is rare suggesting that this constitutes a species level in taxonomy.

Few studies have looked at the incidence and health impact of NoV infection at the community level. The most extensive data are from the UK (Tompkins et al., 1999; Wheeler et al., 1999) and the Netherlands, where a randomised sample of the community participated in cohort studies of infectious intestinal disease (IID). The incidence of community-acquired IID was calculated as 190 per 1000 person years in the UK and 283 per 1000 person years in The Netherlands (de Wit et al., 2001; Tompkins et al., 1999). Viruses were the most frequently identified causes of community acquired gastroenteritis, with NoV detected in 11% of cases in The Netherlands and 7% in the UK. This difference may partly result from the different methods used for virus detection: The group in the Netherlands used RT-PCR whereas the study in the UK employed the far less sensitive electron microscopy, this was confirmed by the recent retesting of stored stool samples from the study (Tompkins et al., 1999). Smaller studies in selected patient populations have been conducted elsewhere, and show that NoV are known to occur as a prominent cause of illness in countries throughout Europe, the USA, Australia, Hong Kong and Japan (Fankhauser et al., 2002; Fankhauser et al., 1988; Iritani et al., 2003; Lau et al., 2004a; Lopman et al., 2004; Lopman et al., 2003; Marshall et al., 2003). Additionally, evidence is mounting that the disease may be common in countries with different degrees of development across the world (Farkas et al., 2002; Gallimore et al., 2004a; Girish et al., 2002; Martinez et al., 2002; Parks et al., 1999; Phan et al., 2004; Reuter et al., 2002). NoV infection is common in all age groups but the incidence is highest in young children (<5 yrs). In recent years, the incidence of norovirus outbreaks has increased with the emergence of a particular variant (Lopman et al., 2004).

Probably the best known presentation of NoV is that of large outbreaks of vomiting and diarrhoea, that lend the disease the initial description of “winter vomiting disease” (Mounts et al., 2000). Since the development of molecular detection methods NoV have emerged as the most important cause of outbreaks of gastroenteritis in institutional settings (i.e. hospitals, nursing homes). The majority of NoV gastroenteritis cases results from direct person-to-person transmission. However, NoV related outbreaks have been shown to be food- or waterborne, caused by for example, contaminated shellfish (Doyle et al., 2004; Kingsley et al., 2002b; Le Guyader et al., 2003), raspberries (Ponka et al., 1999) or drinking water (Carrique-Mas et al., 2003; Kukkula et al., 1999; Parshionikar et al., 2003). Additionally, environmental spread of NoV was found, for instance by contaminated carpets in hotels (Cheesbrough et al., 2000), toilet seats and door handles in a rehabilitation centre (Kuusi et al., 2002), and contaminated fomites on hard surfaces, carpets and soft furnishings in a concert hall (Evans et al., 2002).

A challenging question is how much disease caused by noroviruses can be attributed to foodborne spread. It is clear that the major mode of transmission for noroviruses remains person-to-person (de Wit et al., 2003; Fretz et al., 2005; Karsten et al., 2009; Pajan-Lehpaner and Petrak, 2009). Due to the high rate of secondary transmissions, small initial foodborne events may rapidly present like person-to-person outbreaks, if the initial introduction event was not recognized. In The Netherlands, approximately 12-15% of community cases of NoV gastroenteritis were attributed to foodborne consumption, based on analysis of questionnaire data, and this has been used in later burden of disease estimates. This makes NoV as common a cause of foodborne gastroenteritis as *Campylobacter*, and a more common cause than *Salmonella* (de Wit et al., 2003). In studies of outbreak reports, the term “foodborne” has been used loosely and not standardised. In the EFSA/ECDC Community Summary

Report, outbreaks were stratified into possible and verified foodborne outbreaks, where epidemiological evidence for a food source, or detection of the pathogen in food is considered as evidence. When applying this, only 17% of reported outbreaks are confirmed. This differs greatly for different pathogens, e.g. 26% of *Salmonella* outbreaks are confirmed, but only 4 and 5% of *Campylobacter* and NoV outbreaks, respectively. This may reflect differences in the ability to detect pathogens in food items. A systematic analysis of reported outbreaks of norovirus in a collaborative research project including 13 countries between 2000 and 2007 found evidence for internationally linked diffuse foodborne outbreaks involving approximately 7% of reported outbreaks (total reported 5499). This constitutes a 17.5 fold increase over the previously recognised number, involving 0,4% of outbreaks. The analysis required the availability of both epidemiological and laboratory data, hence limiting it to only 27% of reported outbreaks in this network (Verhoef et al., 2011). Routine harmonised surveillance of viral outbreaks, and surveillance of virus occurrence in food commodities, in combination with systematic strain typing, would be recommended to aid source attribution studies.

2.2. Hepatitis viruses

Four hundred years B.C., Hippocrates described an illness characterized by episodes of jaundice that could probably correspond to a viral hepatitis. Two thousand three hundred years later, at the beginning of the 20th century, the term “infectious hepatitis” was defined and associated to a kind of infectious jaundice occurring in epidemics. In the early 40’s two separate entities were identified “infectious” and “serum” hepatitis, and from 1965 to nowadays the major etiological agents (hepatitis A, B, C, D and E viruses) of viral hepatitis have been identified. While all viral hepatitis are infectious the previously “infectious” and “serum” terms refer to the mode of transmission. The “infectious” type corresponds to those hepatitis transmitted through the faecal-oral route, or enteric hepatitis, and the “serum” hepatitis to those parenterally transmitted. The enteric hepatitis includes two types: hepatitis A and E which can be foodborne and waterborne.

2.2.1. Hepatitis A virus (HAV)

The etiological agent of hepatitis A is the hepatitis A virus (HAV) which belongs to genus *Hepatovirus* within family *Picornaviridae*, and as such it consists of a non-enveloped icosahedral capsid of around 30 nm in diameter containing a positive ssRNA genomic molecule of 7.5 Kb (Fauquet et al., 2005). The genome contains a single open reading frame (ORF) encoding a polyprotein of around 2,225 amino acids preceded by a 5’ non-coding-region (5’NCR) that makes around 10% of the total genome, and followed by a much shorter 3’NCR that contains a poly(A) tract (Baroudy et al., 1985; Cohen et al., 1987). This genome is uncapped but covalently linked to a small viral protein (VPg) (Weitz et al., 1986). The singly translated polyprotein is subsequently cleaved into 11 proteins through a cascade of proteolytic events brought about mainly by the viral 3C protease (Schultheiss et al., 1995; Schultheiss et al., 1994). HAV is a unique picornavirus with many differences in its molecular biology including both its incapacity to induce the inhibition of the cellular protein synthesis and a highly biased and deoptimized codon usage with respect to the cell (Aragones et al., 2008; Borman et al., 1997; Jackson, 2002; Sanchez et al., 2003b). The final goal of this intriguing strategy seems to be the need for a fine-tuning control of the translation kinetics, particularly at the capsid coding region, and the underlying mechanism is the use of a right combination of common and rare codons to allow a regulated ribosome traffic rate thus ensuring the proper protein folding (Aragones et al., 2008; Aragones et al., 2010; Sanchez et al., 2003b). Capsid folding is critical to warrant a high environmental stability for a virus transmitted through the faecal-oral route with long extracorporeal periods.

A single serotype of HAV has been so far reported, being another striking difference with other picornaviruses. In spite of the low antigenic variability of HAV, a certain degree of nucleotide variability, similar to that of other picornavirus, exists and as many RNA viruses HAV occurs as a swarm of mutants termed quasispecies (Domingo et al., 2006; Sanchez et al., 2003a). HAV genomic diversity allows its differentiation into several genotypes and subgenotypes. Different genomic regions, mainly from the capsid coding region (P1) or the junction between the capsid region (P1) and the contiguous non-structural region (P2), have been used to differentiate the genotypes. Particularly,

the carboxi-terminus of the VP3 structural protein, the amino-terminus of the VP1 structural protein, the VP1X2A junction, the region spanning the carboxi-end of VP1 till the amino-terminus of 2B (VP1/P2B), and finally the entire VP1 region (see the review of (Nainan et al., 2006)). However, partial genomic sequences will never guarantee the reliability of the complete P1/2A region. As a matter of fact the identification of some HAV antigenic variants affecting residues not included in the genotyping regions (Costa-Mattioli et al., 2002; Gabrieli et al., 2004; Sanchez et al., 2002) could have been elusive in such circumstances.

The use of long genomic regions has recently been recommended (Costa-Mattioli et al., 2002) for a broad molecular typing of HAV. Nevertheless, the VP1X2A junction is still the genomic region most in use worldwide (Robertson et al., 1992). In this region, seven genotypes were initially defined, whose genetic distance was >15% nucleotide variation. After refining this classification through the addition of more sequences, only six genotypes exist at the present time (Costa-Mattioli et al., 2002; Lu et al., 2004). Three out of these six genotypes (I, II and III) are of human origin while the others (IV, V and VI) are of simian origin. Genotypes I and II contain subgenotypes (Ia, Ib, IIa and IIb) defined by a nucleotide divergence of 7-7.5%.

HAV is a highly stable virus, able to persist for extended times in the environment (Abad et al., 1994a; Abad et al., 1994b; Sobsey et al., 1988) and its transmission by contaminated foods and drinking water has been demonstrated (Bosch et al., 1991; Dentinger et al., 2001; Pinto et al., 2009; Reid and Robinson, 1987; Roseblum et al., 1990; Sanchez et al., 2002), although most cases seem to occur through person-to-person transmission. Foods of primary importance are those susceptible to be contaminated at the pre-harvest stage such as bivalve molluscs, particularly oysters, clams and mussels, salad crops, as lettuce, green onions and other greens, and soft fruits, such as raspberries and strawberries. All these types of food have been implicated in foodborne HAV outbreaks (CDC, 1997; Halliday et al., 1991; Pinto et al., 2009; Shieh et al., 2007; Wheeler et al., 2005) and should be considered the principal targets for virological analysis. However, in approximately 40% of the reported cases of hepatitis A the source of infection cannot be identified (Bosch and Pinto, 2010).

The first documented shellfish-borne outbreak of “infectious hepatitis” occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption (Roos, 1956). However, the most significant outbreak of HAV infection occurred in Shanghai, China, in 1988, in which almost 300,000 cases were caused by consumption of clams harvested from a sewage-polluted area (Halliday et al., 1991). In fact, this is so far the largest virus-associated outbreak of food poisoning ever reported. Depurated shellfish have been associated with outbreaks of norovirus, hepatitis A gastroenteritis, and other viral diseases (Conaty et al., 2000).

The distribution patterns of hepatitis A in different geographical areas of the world are closely related to their socioeconomic development (Gust, 1992; Hollinger and Emerson, 2007; Previsani et al., 2004). The endemicity is low in developed regions and high in underdeveloped countries. The epidemiological pattern has important implications on the average age of exposure and hence, as above stated, on the severity of the clinical disease. Since hepatitis A infection induces a life-long immunity (Hollinger and Emerson, 2007), severe infections among adults are rare in highly endemic regions where most children are infected early in life. In contrast, in low endemic areas the disease occurs mostly in adulthood, mainly as a consequence of travelling to endemic regions, having sexual risky practices or consuming contaminated water or food; and hence the likelihood of developing severe symptomatic or fatal illness is high. An epidemiological shift, from intermediate to low prevalence, has been noticed in recent decades in many countries, particularly in Southern Europe, including Spain, Italy and Greece (Dominguez et al., 2008; Germinario et al., 2000; Van Damme and Van Herck, 2005). Consequently, the Mediterranean basin as a whole should no longer be considered as an endemic area (Pinto et al., 2007; Previsani et al., 2004).

Additionally, some other countries from Eastern Europe (Cianciara, 2000; Tallo et al., 2003) have also described significant declines in the incidence of hepatitis A. Likewise, in several Asian and American

countries a shift from highly to moderate endemic has as well been described (Barzaga, 2000; Tanaka, 2000).

2.2.2. Hepatitis E virus (HEV)

HEV is a non-enveloped icosahedral virus with a diameter of 35 nm, classified into the unassigned genus *Hepevirus*. The genome consists of one single-stranded RNA molecule of positive polarity and about 7 kb in length. The major ORFs are ORF-1, which encodes a non-structural polyprotein, ORF-2 encoding the capsid protein and ORF-3 encoding a phosphoprotein. The HEV strains can be grouped into 4 genotypes, with different geographical distribution and host range. Genotype 1 is endemic in Asia and Africa and genotype 2 is endemic in Mexico and western Africa. Whereas these genotypes have been found exclusively in humans; genotypes 3 and 4 have also been detected in pigs and other animal species. Genotype 3 is distributed worldwide and genotype 4 is restricted to Southeast Asia. Thus the endemic strains found in Europe are usually of genotype 3. In addition to the HEV genotypes 1 to 4, distinct HEV-like viruses with lower sequence identity to the human strains have been detected in chicken and rats.

The epidemiology of HEV is complex, and a foodborne transmission of HEV from animal products to humans is an emerging concern. Several studies suggest the following food items as risk factors for acquisition of HEV infection: pork pies, liver pate, wild boar, under-cooked or raw pork, home-made sausages, meat (in general), unpasteurized milk, shellfish and ethnic foods [references in (Lewis et al., 2010)]. However, only very few systematic studies have been performed so far; therefore, nearly none of these risk factors is sufficiently substantiated. One systematic case-control study has been performed in Germany, in which eating of any offal or wild boar meat was identified as risk factor for autochthonous hepatitis E (Wichmann et al., 2008). In addition, another recent small-scaled case-control study identified eating of raw pig liver sausage as a risk factor for hepatitis E in France (Colson et al., 2010). Previous publications from Japan indicate direct HEV transmission by eating raw or undercooked meat from wild boar or deer by detailed analysis of small outbreaks (Li et al., 2005; Tei et al., 2003).

No detailed information on hepatitis E cases, including the proportion of foodborne cases, is available for the EU. Worldwide, it has been estimated that approximately 2 billion people have been exposed to HEV (Aggarwal and Jameel, 2008). However, the vast majority of hepatitis E cases are recognized in the endemic regions in Asia, Africa and Central America, where transmission is mainly due to faecally contaminated water. Europe is not an endemic region, but sporadic hepatitis E cases have been described in France, The Netherlands, Spain, Hungary, the UK, Denmark, Norway (Teo, 2009), indicating an EU-wide distribution of the virus. In Germany, where hepatitis E cases are notifiable since 2001, a total of 40 to 220 cases per year are registered, with increasing tendency¹⁰. About 2/3 of these cases are not linked to travels into the endemic regions and therefore recognized as autochthonous infections (Wichmann et al., 2008). Although the consumption of offal and wild boar meat has been identified as a risk factor for the German hepatitis E cases (Wichmann et al., 2008), the proportion of foodborne cases is not known. In France the disease is also notifiable and 218 cases have been identified in 2008. Among these cases 146 have been identified as autochthonous cases, 23 to travels and no epidemiological data was available for 49 cases (Nicand et al., 2009).

HEV is associated with large outbreaks of hepatitis E among humans in endemic countries. This predominantly includes inhabitants from Asian and African countries, which are exposed to the virus due to poor sanitary conditions (Purcell and Emerson, 2001). Sewage overflow that results from heavy rainfall may contaminate surface water that is used for drinking water production or as source for water used for household tasks. As water is widely distributed and used, the number of people exposed is generally large, explaining the large-scale outbreaks of HEV in developing countries (Viswanathan, 1957).

¹⁰ Robert Koch-Institut: SurvStat, <http://www3.rki.de/SurvStat>, March 2010.

Although hepatitis E outbreaks are only observed in developing countries, anti-HEV antibodies have been found globally, including in industrialized countries. The reported human anti-HEV immunoglobulin G seroprevalence among the general population in industrialized countries ranges from 2.3 to 33% , but direct comparison of data from different studies is challenging because diagnostic approaches are not standardised (Lewis et al., 2010). Some of the HEV infections in industrialized countries are attributed to travel into HEV endemic areas, but an increasing number of non-travel-related cases have been reported (Lewis et al., 2010).

Four transmission routes have been reported for HEV: (i) faecal-oral transmission due to contamination of drinking water, (ii) foodborne transmission, (iii) transmission by transfusion of infected blood products, and (iv) vertical (materno-fetal) transmission (Aggarwal and Naik, 2009). Direct horizontal transmission of HEV between humans is unusual. The distinct transmission routes for HEV in Europe are unknown; however, several risk factors for autochthonous hepatitis E in Europe have been suggested (see above).

Zoonotic spread of HEV has been suspected and several animal species have been identified as potential virus reservoirs (Teo, 2009). This predominantly includes domestic pigs and wild boars, in which both HEV-specific antibodies and HEV genome sequences have been detected repeatedly. Most of these sequences are closely related to human HEV sequences (Lewis et al., 2010). The prevalence of HEV in pigs and wild boars in Europe as assayed by PCR ranges from 5.9% to 76% and 3.8% to 25%, respectively (Lewis et al., 2010). HEV sequences closely related to human HEV have also been detected in some species of deer (Teo, 2009). An HEV strain has also been recently detected in farmed rabbits in China (Zhao et al., 2009). In Japan, several cases of hepatitis E have been linked epidemiologically to eating undercooked pork liver or wild boar meat (Masuda et al., 2005; Matsuda et al., 2003; Yazaki et al., 2003). Most direct evidence of zoonotic HEV transmission was obtained when four cases of hepatitis E were linked directly to eating raw deer meat by the presence of identical HEV strains in the consumed deer meat and patients (Tei et al., 2003). Furthermore, zoonotic transmission of HEV genotype 3 from wild boar to human was demonstrated by nucleotide sequence identity in HEV isolated from a patient and the wild boar meat she consumed (Li et al., 2005).

Other animal species, in which HEV-related agents have been identified by genome sequencing, include chicken and rats. The avian HEV detected in chicken is only distantly related to human HEV, has been shown to be unable to infect monkeys and is therefore considered to be non-transmissible to humans (Huang et al., 2004). After many reports showing the presence of HEV-specific antibodies in several rat species, genomic sequences of an HEV-related virus were recently identified in Norway rats (Johne et al., 2010). The zoonotic potential of the rat HEV is not known so far. In addition to these animal species, serological data suggest the presence of HEV-related agents in cattle, horses, and some pet animals (Teo, 2009).

Risk factors for hepatitis E and HEV infection in the industrialized countries have been investigated in several studies and recently systematically reviewed (Lewis et al., 2010). From this review, a general trend for men and older people for developing acute hepatitis E is evident. In addition, co-morbidity, e.g. underlying chronic liver disease, liver cirrhosis or a history of high alcohol consumption, is connected with the development of hepatitis E. Direct contact to animals is considered as a risk factor for HEV infection (Presence of HEV-specific antibodies), however, a significant correlation with hepatitis E cases is not evident from the studies so far. Other risk factors including contact to human sewage, water exposure, or a parental transmission, e.g. through blood transfusions, have been suggested, but are not conclusively supported by the analyzed studies.

Data are missing on the incidence of hepatitis E in EU countries. Also, the distinct transmission pathways of HEV and especially the proportion of foodborne cases out of total hepatitis E cases are not known.

2.3. Viruses occasionally reported as foodborne

Outbreaks associated with foodborne transmission of newly emerging viruses are a low probability event but have a potentially high impact. The examples of SARS and avian influenza, and the relatively uncontrolled foodborne transmission of less dangerous viruses (e.g. noroviruses), illustrate that, should a novel pathogen with efficient foodborne transmission arise, we are likely to be ill prepared to handle such an event. SARS coronavirus was spread into the human population through the preparation and consumption of food of animal origin, which appears to have contracted the infection from another reservoir, probably bats (Lau et al., 2004b). Infectious H5N1 avian influenza virus has been cultured from duck meat, and the consumption of duck blood has resulted in the infection of humans (Tumpey et al., 2003). Recent Opinions from the BIOHAZ Panel in EFSA^{11, 12}, reviewed the food safety aspects of avian influenza and of novel influenza virus H1N1, concluding that foodborne infection with avian influenza is unlikely but can not be ruled out entirely, and that food contaminated with nH1N1 influenza viruses does not appear to be a vehicle for infection in humans. SARS coronavirus and related viruses have been found in bat populations, also in Europe, but this does not at present constitute a significant risk for foodborne transmission (Drexler et al., 2010). Similarly, H5N1 infections of humans are rare, and most frequently associated with direct contact with ill poultry. A third pathogen considered to be of concern in the WHO expert meeting are Nipah viruses, following observations of infection of humans following consumption of fruits contaminated with Nipah virus through saliva of fruit bats (Luby et al., 2006). Again, here the biggest concern is the possible adaptation of these viruses to humans, as they are related to known viruses that are among the most transmissible human viruses that emerged from the animal world (e.g. measles).

For Europe, infections with flaviviruses may be relevant. Viruses belonging to the *Flavivirus* genus are mainly arthropod borne viruses but examples of zoonotic foodborne transmission have been reported. The tick borne encephalitis viruses (TBEV) are transmitted from their natural hosts, mostly rodents, by ticks (*Ixodes sp*) to humans, or for example to cows, sheep and goats. In these animals the viruses can be shed via milk and consumption of contaminated raw milk can lead to infection and a disease described as “biphasic milk fever” in humans. Moreover, infectious TBEV is found in yoghurt, butter and cheese and the viruses are able to survive in gastric juice for 2 hours. The high resistance to acid is not concomitant with a generalized high resistance to inactivation. Due to the lipid envelope, TBEV is readily inactivated by heat treatment, detergents and organic solvents. Even though a viremic phase is common during a TBEV infection in several animal species, foodborne infection via contaminated meat or organs is unlikely due to the fast virus inactivation at elevated temperatures. TBEV can produce a variety of clinical symptoms after an incubation period of 7 to 14 days. Common early symptoms are fatigue, headaches, and pain in neck, back and shoulders. These may progress into a sudden onset of the classical symptoms such as fever, nausea and vomiting, severe muscle pain in neck, back, shoulders and limbs, and encephalitis. The case fatality rate in Europe is in general low (0.5-1.5%) but may differ per virus strain and/or geographic region.

While the above examples of “emerging infections” could lead to the conclusion that the risk of foodborne transmission can be considered negligible, they have caused quite some concern because evidence to support this claim is lacking. This was again problematic when filovirus particles were identified in asymptomatic pigs in the Philippines recently, another illustration of the difficulties in risk assessment for such situations.

There is consensus among virologists that the probability of the emergence of new viruses or the evolution of old viruses into new forms is inevitable, given the demographic, economical, and sociological changes that we are now facing. Therefore, having mechanisms in place to rapidly

¹¹ Statement on Food safety considerations of novel H1N1 influenza virus infections in humans. EFSA Panel on Biological hazards (BIOHAZ). <http://www.efsa.europa.eu/en/scdocs/doc/1629.pdf>.

¹² Scientific Report of the Scientific Panel on Biological Hazards on “Food as a possible source of infection with highly pathogenic avian influenza viruses for humans and other mammals” <http://www.efsa.europa.eu/en/scdocs/scdoc/74r.htm>.

address the probability and possible consequences of foodborne transmission of a new infectious disease when it emerges should be a priority.

3. Hazard characterisation

3.1. Norovirus

Human norovirus infection is popularly known as ‘winter vomiting disease’, because of the observation that disease outbreaks follow a pattern of winter-seasonality: outbreaks in the Northern hemisphere are most common between November and March. In the Southern hemisphere a similar seasonal pattern has been observed in certain countries (Australia), but not in others (New Zealand). Illness caused by noroviruses is also known as the ‘gastric flu’ or ‘stomach flu’. Outbreaks with high media-impact among vacationers on cruise ships have also yielded the name ‘cruise ship virus’.

The illness caused by norovirus is usually described as mild and self-limiting. Incubation time is typically 12-72 h and symptoms may last 1-3 days, although longer times up to 5 days have been reported, particularly in young children and the elderly. Diarrhoea is the most commonly reported symptom, followed by vomiting, abdominal pain, cramps, nausea and fever. The diarrhoea is watery, rarely containing mucus and blood. In people with co-morbidity or in the elderly, illness may be more severe and sometimes has very serious consequences, such as prolonged infections and excess mortality. Chronic NoV infection has recently been recognized, and may be much more common than previously recognized. In a retrospective study of hospitalized patients who acquired NoV infection, 15% of persons for whom follow-up was done developed chronic NoV infection (Beersma et al., 2009).

The study of duodenal biopsies of norovirus-infected people provided a basis for understanding the cause of diarrhoea, namely a combination of epithelial barrier dysfunction in the duodenum, a reduction of tight junctional proteins, increased apoptosis in duodenal epithelial cells and increased anion secretion. Abdominal computed tomography (CT) scans of children with acute norovirus infections revealed wall thickening and enhancement in the different parts of the small intestine, namely the duodenum, jejunum and ileum, as well as fluid filled bowel loops. Recently, a bowel perforation of the small bowel resulting of norovirus infection was reported.

NoV is shed in high quantities in the stool of infected persons; around 10^8 but up to 10^{11} RNA copies per gram of stool were reported for different GI and II viruses (Atmar et al., 2008). Projectile vomiting, which is a very typical symptom for norovirus illness, is thought to contribute to spread of the viruses by environmental contamination through dispersal of droplets generated while vomiting.

Shedding of virus continues after clinical recovery of the patient, and may last three or four weeks in otherwise healthy people, but can be especially long in young children. In a hospital study involving people of all ages, higher concentrations of virus in stool were found to be associated with older aged patients and also with prolonged diarrhoeal symptoms and increased severity of symptoms. In immuno-compromised patients severely prolonged illness accompanied by prolonged shedding may last up to several years. Teunis et al., (2008) used the results of volunteer studies with GI.1, Norwalk virus, to estimate probability of infection of a single norovirus particle. This was extremely low, with a probability of infection after exposure to 1 particle of 0.5, and the ID₅₀ at 18 virus particles. The probability of becoming infected increases with the dose, as was observed in volunteer studies and during outbreaks (de Wit et al., 2007; ter Waarbeek et al., 2010; Teunis et al., 2008; Visser et al., 2010).

Eventhough there is no classical virological proof of the existence of different serotypes of norovirus by classical virus neutralization methods, the genetic diversity displayed by noroviruses likely translates into antigenic diversity, so that infection with a strain of one genotype may not confer immunity against strains of another genotype or even variants within a genotype. Furthermore, volunteer studies have shown that protective immunity after infection may be absent or short-lived (Parrino et al., 1977). The combination of antigenic diversity and the apparent lack of long term

protective immunity are the likely cause of the occurrence of norovirus infections in children, adults and the elderly. In effect one individual may suffer repeated infections, even with viruses belonging to the same genotype and therefore people of all ages are affected by norovirus illness, unlike with e.g., rotavirus, where re-infection only occurs when a different serotype is encountered.

Differences in host-susceptibility for different genotypes have been reported, and are based on the presence or absence of specific virus receptors in the potential host. Although additional research is needed to establish more detail and to clear up some controversies, the currently proposed receptors are encoded by the human histo-blood group antigen (HBGA) genes. The HBGA system is controlled by multiple gene families. Polymorphisms in the genes encoding these antigens or proteins that have a role in their biosynthesis have been demonstrated, resulting in differences in susceptibility of subgroups in the population for noroviruses. However, the exact nature of interaction of noroviruses with their host is strain dependent, precluding general statements about differences in susceptibility.

3.2. Hepatitis A virus (HAV)

After replication in the liver, hepatitis A virus (HAV) is found in the bile in large quantities, reaching the intestines by the bile duct and being subsequently shed in feces. Virion stability of HAV in the presence of biliary salts is guaranteed by the absence of a lipid envelope, which is not the case for serum hepatitis viruses. Symptomatic individuals as well as asymptomatic carriers shed virus that may contaminate water and food. HAV concentration in the patient stools is highest (up to 10^{11} genome copies/g of feces) after two weeks of the onset of symptoms and lasts at least four more weeks. An additional concern is that viral excretion even in symptomatic patients starts before the onset of symptoms. Hepatitis A infection is mainly propagated via the faecal-oral route being the person-to-person contact the most common mode of transmission. In fact HAV persistence in contaminated fomites, such as sanitary paper, sanitary tile and latex gloves, is very long (Abad et al., 1994a). In consequence, given the high excretion level of HAV, transmission of the infection is facilitated when poor sanitary conditions occur. In addition, active homosexual men are a risk group for HAV transmission and outbreaks are frequently reported (Stene-Johansen et al., 2002; Stene-Johansen et al., 2007; Tortajada et al., 2009). Transmission through the parental route may also occasionally occur (Noble et al., 1984; Sheretz et al., 2005).

Hepatitis A infection mostly develops asymptotically or subclinically among young children (under 5), while in older children and in the adulthood the infection usually proceeds with symptoms (Previsani et al., 2004). In this latter case, the clinical course of hepatitis A is indistinguishable from that of other types of acute viral hepatitis. The clinical case definition for hepatitis A is an acute illness with moderate onset of symptoms (fever, malaise, anorexia, nausea, abdominal discomfort, dark urine) and jaundice, and elevated serum bilirubin and aminotransferases levels later on. The HAV infectivity is unknown but according to the US Food and Drug Administration presumably is around 10-100 virus particles¹³.

The incubation period of hepatitis A ranges from 15 to 50 days and clinical illness usually does not last longer than 2 months, although 10%-15% of patients have prolonged or relapsing signs and symptoms for up to 6 months (Glikson et al., 1992; Sjogren et al., 1987). In fact, with the advent of new highly sensitive techniques even in normal clinical courses a high and long lasting viremia has been detected (Costafreda et al., 2006), with the peak (up to 10^7 genome copies/ml of sera) occurring at two weeks after the onset of symptoms and lasting up to an average of six weeks after the start of symptoms (Bower et al., 2000; Costafreda et al., 2006). There is no evidence of chronicity of the infection, however, occasionally the infection may proceed to a fulminant hepatitis, mainly among patients with underlying chronic liver diseases (Akriviadis and Redeker, 1989; Previsani et al., 2004).

¹³www.fda.gov/food/foodsafety/foodborneillness/foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/ucm071294.htm

Although it is generally accepted that the severity of hepatitis A is mostly related with host factors such as aging and the occurrence of other underlying liver diseases, viral factors may also play a role in pathogenesis. Among these viral factors it may be pointed that some mutations at the 5'NCR of HAV or at the VP1X2A and 2C regions have been associated with fulminant hepatitis (Fujiwara et al., 2002; Fujiwara et al., 2001; Fujiwara et al., 2003) or higher virulence in tamarinds (Emerson et al., 2002), respectively. However, there is no consensus whether the VP1X2A-derived genotypes are clinically different, although some strains belonging to the former genotype VII now included in genotype II were associated with fulminant cases (Ching et al., 2002; Costa-Mattioli et al., 2002; Mackiewicz et al., 2010).

In addition to the clinical implications of genetic variability, genotype characterization may be highly relevant to trace the origin of outbreaks. However, when typing outbreak-related isolates, it must be borne in mind that not always an identical nucleotide sequence is obtained from a putative source virus (e.g. contaminated food or water) and the virus found in the infected recipients. High mutation rates render very unlikely the complete conservation of sequences as soon as virus replication occurs, in this case in the infected individuals.

3.3. Hepatitis E virus (HEV)

Human infections by HEV can lead to clinical disease, referred to as hepatitis E. The incubation period in human volunteers after oral infection is 4 to 5 weeks; more variable incubation periods of 2 to 10 weeks have been reported during hepatitis E outbreaks (Aggarwal and Naik, 2009). Clinical symptoms of hepatitis E in humans cannot be distinguished from the symptoms of other forms of viral hepatitis. Serologic or molecular evidence is required for the confirmation of a HEV infection as possible cause of the clinical symptoms. The general symptoms of hepatitis are anorexia, jaundice and liver enlargement (Purcell and Emerson, 2001). Furthermore, about half the patients with hepatitis E display abdominal pain and tenderness, nausea and fever. Hepatitis E is mostly self-limiting and in general does not progress to chronicity (Jameel, 1999; Purcell and Emerson, 2001), although several chronic cases have been reported recently (Gerolami et al., 2008; Haagsma et al., 2008; Kamar et al., 2008). Fulminant hepatitis has been described in some cases. Case fatality rates among patients are generally low between 1 and 5 % (Pavio et al., 2010), but may reach up to 25% in pregnant women for at least genotype 1 (Kumar et al., 2004). Faecal shedding of HEV occurs in most hepatitis E cases for approximately 2 weeks (Takahashi et al., 2007). However, a small group of patients shows prolonged faecal excretion for up to 52 days as assayed by RT-PCR. For one patient, infectious HEV could be isolated from faeces in cell culture at 30 days after the onset of disease and HEV-RNA could be detected by RT-PCR for up to 121 days (Takahashi et al., 2007).

Infected animals do not normally show clinical signs of disease. The natural time course of HEV infection has been predominantly studied for pigs (Pavio et al., 2010). HEV seems to be very effectively transmitted between pigs resulting in a synchronization of the course of infection. HEV infection usually occurs at 8 to 12 weeks of age after the decline of maternal antibodies. Most of the infected pigs show a viraemia at 3 months of age and faecal shedding of HEV between 10 to 16 weeks of age. The immune response as reflected by seroconversion between 14 to 17 weeks of age usually limits the infection; however, a low number of pigs show prolonged shedding after 22 weeks of age. HEV mainly replicates in the liver of infected pigs; 0.8 to 11% pig livers sold in grocery stores in different countries have been shown to contain HEV RNA. The strong age dependence of the course of infection has not been found in wild boars as no significant differences in the HEV RNA detection rates in livers from different age classes were observed in these animals (Schielke et al., 2009).

The dose response relationship for HEV for humans is unknown. A volunteer orally infected with a 10% stool suspension derived from an HEV-infected patient developed clinical signs of hepatitis thus confirming the oral transmission route of HEV in humans (Chauhan et al., 1993). By infection experiments with cynomolgus monkeys HEV infection as determined by seroconversion could be detected after intravenous inoculation of an HEV suspension containing one PCR-detectable genome unit (Tsarev et al., 1994). Although the distinct sensitivity of the nested PCR protocol used for the definition of the PCR-detectable unit is not known, it can be concluded from the experiment, that the

intravenous infectivity of HEV for cynomolgus monkeys is very high. In contrast, in the same study it was shown that oral inoculation of cynomolgus monkeys did not result in infection even after application of 10^5 PCR-detectable genome units. In addition, clinical signs of hepatitis as assayed by significant elevation of ALT activity in the blood were only evident after intravenous inoculation of more than 10^4 PCR-detectable genome units. Taken together, the results of the study suggest that the intravenous route of infection is more efficient than the oral route and that a relative high dose of virus is needed to induce hepatitis by any of the 2 routes. These results are largely confirmed by infection experiments with pigs, although clinical disease can mostly not be induced in these animals (Kasorndorkbua et al., 2004).

Little is known about the factors of pathogenicity of HEV. Generally, the clinical course of hepatitis E is similar regardless the infecting genotype. Recently, two silent mutations present in some of the genotype 4 strains have been linked to increased disease severity and the induction of fulminant hepatitis (Inoue et al., 2009). The distinct reasons for the high mortality rates of hepatitis E in pregnant women are unknown, although several immunological and hormonal mechanisms have been proposed (Chandra et al., 2008).

Epidemiological observations during hepatitis E outbreaks suggest that people previously infected with HEV are protected against further disease (Aggarwal and Jameel, 2008). The duration of a protective antibody response following HEV infection is unknown. Anti-HEV IgG has been described to disappear within 6 months to 4 years; however, one study reported the persistence of such antibodies for up to 14 years in about half of the people infected during an outbreak of hepatitis E (Aggarwal and Jameel, 2008). Anti-HEV IgM occurs early in the disease, usually by the time of the onset of clinical symptoms, and disappears after several months (Purcell and Emerson, 2001). Therefore, IgM is widely used as a diagnostic parameter confirming acute hepatitis E infection. A vaccine against hepatitis E is not commercially available so far, although several vaccine candidates, mainly based on the recombinantly expressed capsid protein, are currently tested with promising results (Aggarwal and Jameel, 2008).

Data gaps include missing knowledge about the dose response relationship of HEV and factors influencing pathogenicity of HEV strains as well as reasons for severe hepatitis E cases.

4. Exposure assessment

4.1. Natural persistence (resistance to different physical/chemical factors)

Transmission of a virus is dependent not only on its interaction with a host, but on its interaction with the environment outside of the host. Viruses are obligate intracellular parasites, which have an absolute requirement for a host organism in order to replicate themselves. Unlike bacteria, they possess no intrinsic metabolism, and can not replicate outside a host. If they contaminate the environment or a foodstuff, their numbers will not increase, and will only remain stable or decline from the original contaminating load. Conversely, they do not require nutrients to survive, unlike bacteria. The term “survival” used here means natural persistence of infectious viruses, i.e. when no process (such as heat, chemical disinfection etc.) has been deliberately applied to eliminate them. The longer a virus can persist outside a host, the greater are its chances for transmission between one host and another. Virus survival is affected by various conditions and factors such as temperature, moisture, and pH. Enteric viruses possess a degree of robustness which allows them to remain infectious during various ranges of these conditions that they may encounter in foodstuffs or the environment. This robustness is not shared to the same degree amongst all enteric virus types, with some being able to persist for longer than others in e.g. wetter or dryer environments, and others being more resistant to temperatures increases; but generally, all enteric viruses have a potential for persistence which contributes towards their potential as hazards in the environment or in foods.

Table 2: Factors affecting virus persistence in environmental samples^a

Factor	Effect on viruses
Physical	
Heat	Inactivation is directly proportional to temperature
Light	Light, specially its UV component is germicidal
Desiccation or drying	Usually increased inactivation at lower relative humidity
Aggregation / Adsorption	Protects from inactivation
Pressure	High pressure induces inactivation
Chemical	
pH	Stability is most greatly affected by extreme pH
Salinity	Increased salt concentrations are virucidal
Ammonia	Ammonia salts show virucidal activity
Inorganic ions	Some metal ions (e.g. Pt, Pd, Rh) show virucidal activity
Organic matter	Dissolved, colloidal and solid organic matter protect from inactivation
Enzymes	Proteases and nucleases contribute to inactivation
Biological	
Microbial activity	Contributes to inactivation
Protozoan predation	Contributes to removal
Biofilms	Adsorption to biofilms protects from inactivation, while microbial activity in biofilms may be virucidal

^aStability varies according to the strain and type of virus

Information about factors presented in table 2 may not be available for the different viruses covered by this opinion.

4.1.1. Noroviruses

There is no direct information on the survival of NoV on foods or in the environment. This is because survival studies require the use of infectious virus growing on cultured cells, and so far there is no robust method for the cultivation of human NoV. A recent study (Lamhoujeb et al., 2009) has employed NoV particles directly, although not directly assessing their infectivity. By using a method combining enzymatic digestion of viral RNA with a molecular detection assay, and assuming that non-infectious particles possess damaged capsids which leave the genetic material exposed, the authors inferred that NoV could survive in an infectious state for up to 8 weeks on PVC and stainless steel surfaces at 4° C, and up to 4 weeks at 20° C, dependent on the humidity (high humidity being more conducive to survival).

Most studies on NoV survival have used surrogates such as feline calicivirus (FCV) and murine norovirus (MNV). Cannon et al., (2006) compared the inactivation profiles of MNV-1 to FCV in an effort to establish the relevance of MNV-1 as a surrogate virus, and concluded that the latter was more appropriate due to its ability to tolerate gastric pH levels and its greater genetic relatedness of human NoV. Nevertheless, information gained from the use of FCV as a surrogate may be instructive since the inference could be that NoV could display even more robust survival under the same conditions. Thus, when infectious FCV has been observed (Mattison et al., 2007) to persist on lettuce stored at 4° C and 22.5° C for 3 and 7 days respectively, and on strawberries stored at 4° C and 22.5° C for 3 and 7 days respectively, then it could be expected that NoV could persist in an infectious state for a longer period under such conditions. Baert et al., (2008c) found no reduction of MNV on spinach or onions held at -20° C for 6 months; this is in keeping with observations that from outbreaks that indicate that NoV can survive in frozen produce and remain infectious from the time of processing to the time of consumption (Maunula et al., 2009).

Circumstantial information from outbreaks also reveals that NoV can remain infectious on fresh salad vegetables (Ethelberg et al., 2010; Gallimore et al., 2005) within shellfish (Simmons et al., 2007), and on inanimate surfaces (Cheesebrough et al., 1997) for several days at least. This pattern of survival is mirrored in the information from studies of other virus types (Rzezutka and Cook, 2004), and the

Scientific Committee on Veterinary Measures Relating to Public Health¹⁴ considered it useful to take this information into regard as a determination of the likely survival of NoV in similar conditions. Thus, NoV can be expected to persist up to several weeks on vegetable crops which have been in contact with contaminated sewage or irrigation water, and, on fresh produce under conditions commonly used for storage in households, at least as long as the time generally taken between purchase and consumption. The overall message is that NoV, once it has contaminated a foodstuff at source, could remain infectious long enough for consumption of that foodstuff to constitute a risk to the consumer.

4.1.2. Hepatitis A virus

HAV has been shown experimentally to be able to survive in several environments, such as water, foods and surfaces (Rzezutka and Cook, 2004). HAV can persist for up to 5 hours at pH 1 (Scholz et al., 1989) and can remain viable in faeces after drying for at least 30 days under conditions simulating a typical environmental exposure (McCausland et al., 1982). In other experimental studies, HAV could survive for at least 4 hours on faecally contaminated surfaces, such as stainless steel, and could be transferred from there to fingertips, and back again (Mbithi et al., 1991). Transfer was positively influenced by moisture. Abad et al., (1994a) found that HAV could survive on various materials for at least 60 d. HAV was more generally more resistant to desiccation than other enteric viruses such as adenovirus and poliovirus. The persistence of HAV on environmental surfaces and its ability to transfer to animate environments may be important factors in the spread of this virus, especially in food preparation settings. For instance, cafeteria trays contaminated by an infected food handler, with which food came in direct contact, were the vehicle in at least one foodborne hepatitis A outbreak (Cliver, 1985). Terpstra et al., (2007) studied survival of HAV on stainless steel surfaces. Storage of the contaminated stainless steel at room temperature resulted in less than 1 log₁₀ reduction after 7 days, and virus could still be found on the material after 28 days.

HAV has the ability to survive in seawaters for several weeks (Bosch, 1995; Callahan et al., 1995), with survival being more prolonged in colder temperatures (Bosch, 1995; Crance et al., 1998). This potential promotes their chances of being collected by filter-feeding shellfish. Outbreak investigations have indicated that viruses can persist in shellfish over several weeks following contamination (Conaty et al., 2000; Lees, 2000). In fresh waters, it is possible that HAV could survive for several days with little loss of infectivity. In river waters, little or no decline in infectivity of HAV was observed after 48 d (Springthorpe et al., 1993). In groundwater, HAV could survive longer than 12 weeks, losing only approximately 1 % infectivity during that period (Sobsey et al., 1989). In tap water, HAV survived at various temperatures for up to 60 days (Enriquez et al., 1995). This information indicates that HAV could survive long enough in water, between a contamination event and the use of the water for crop irrigation or during food processing, to constitute a risk to health. Irrigation of crops with contaminated water or organic waste is a potential means of contaminating foodstuffs with enteric viruses, and studies with other enteric virus types, e.g. poliovirus have demonstrated that viruses can be transferred to the surfaces of vegetables and persist there for several days, following the application of sewage sludge or effluent (Rzezutka and Cook, 2004). Stine et al., (2005b) studied the survival of HAV inoculated onto the surface of fruits of cantaloupe, lettuce and bell peppers. In this particular study, survival was significantly longer on cantaloupe than on lettuce or bell peppers, and virus survived better in conditions of low relative humidity.

Once on foodstuffs such as vegetables, HAV can persist under normal storage conditions over the periods usual between purchase and consumption. Croci et al., (2002) evaluated HAV survival on carrot and fennel. Package was not specified in the paper. The carrots and fennel were cut into small pieces, inoculated with HAV by draining, afterwards strained, lay to dry and divided into aliquots of 20 g. On these vegetables, a more pronounced decline in HAV infectivity was observed, with complete inactivation of HAV by day 4 for carrot and by day 7 for fennel. It was considered that this

¹⁴ Opinion of the Scientific Committee on Veterinary Measures Relating to Public Health on Norwalk-like viruses, 2002. http://ec.europa.eu/food/fs/sc/scv/out49_en.pdf

may have been due to the presence of antimicrobial substances in these vegetables. The inference from several outbreaks of hepatitis A implicated to frozen fruit (Hutin et al., 1999; Ramsay and Upton, 1989) is that HAV can survive for several months in frozen foods.

4.1.3. Hepatitis E virus

Only limited information is available about the physical stability of HEV, mainly due to the lack of an efficient, rapid and sensitive cell culture system for detection of infectious virus. All of the published tissue culture systems rely on a high amount of virus for infection and the assessed thermal stability seems to be dependent on the cell culture system as well the HEV strain used. Studies on natural persistence of HEV are missing.

4.2. Effects of treatments used in food processing on viruses

Foodborne viruses such as NoV and HAV are quite persistent as indicated above in the environment and in foods. In contrast to most microbiological agents, viruses cannot grow on food and thus the contamination level cannot increase during processing or storage but survival should be considered due to a high infectivity (Carter, 2005; Koopmans and Duizer, 2004). The effect of food processing treatments on NoV and HAV is therefore discussed in the sections below. In the first place, the effect of acidification on microbial growth inhibition is described. Secondly, the use of preservation methods for microbial inactivation (such as heat treatment, high hydrostatic pressure processing and irradiation) to eliminate viruses is discussed and presented (see also table 3.) Finally, the efficacy of decontamination methods on fresh produce (see also table 4), and purification procedures applied on live bivalve shellfish to reduce the viral load is included.

Table 3: The efficacy of heat treatment, high hydrostatic pressure processing and irradiation to inactivate foodborne viruses

Viruses	Inactivation method	Matrix	Log reduction ^a	Reference
Heat treatment				
<i>Reoviridae</i>	RoV ^b	60°C 10 min	Cell culture medium	7 (Mahony et al., 2000)
	HAV ^c	85°C <0.5 min	Milk	5 (Bidawid et al., 2000a)
<i>Picornaviridae</i>		71°C 6.55 min (skimmed); 8.31 min (homogenized); 12.67 min (cream)	Milk	4; 4; 4
	HAV	85°C 0.96 min (28°Brix); 4.98 min (52°Brix)	1 g strawberry mashes	1; 1 (Deboosere et al., 2004)
	HAV	80°C 8.94 min (52°Brix)		1
	HAV	60°C 10 min; 80°C 3 min	4 ml virus suspension	> 4.6; > 4.6 (Crocchi et al., 1999)
	Poliovirus	60°C 10 min; 80°C 3 min	4 ml shellfish homogenate	2; 2
		72°C 0.25 min; 72°C 0.5 min	Milk	0.56; >5 (Strazynski et al., 2002)
		42°C 30 h; 55°C 30 min	Yoghurt	0.41; >5
		Steaming 30 min	Oysters	2 (Di Girolamo et al., 1970)
<i>Caliciviridae</i>	FCV ^d , CaCV ^e	71.3°C 1 min	Cell culture medium	3 (Duizer et al., 2004)
	FCV, CaCV	37°C 24 h; 56°C 8 min	Cell culture medium	3; 3
	FCV	0.5 min immersion of 6-8 cockles in boiling water	Cockles	1.7 (Slomka and Appleton, 1998)
	FCV	56°C 3 min; 56°C 60 min	Cell culture medium	No red ^g ; 7.5 (Doultree et al., 1999)
		70°C 1 min; 3 min; 5 min	Cell culture medium	3; 6.5; 7.5
		Boiling 1 min	Cell culture medium	7.5
	FCV	70°C 1.5 min	Cell culture medium	6 (Buckow et al., 2008)
	FCV	63°C 0.41 min; 72°C 0.12 min	Cell culture medium	1; 1 (Cannon et al., 2006)
	NoV	60°C 30 min		Incomplete (Dolin et al., 1972)
	MNV-1 ^f	63°C 0.44 min; 72°C 0.17 min	Cell culture medium	1; 1 (Cannon et al., 2006)
High hydrostatic pressure processing				
<i>Reoviridae</i>	RoV	300 MPa, 25°C, 2 min	Cell culture medium	8 (Khadre and Yousef, 2002)
	HAV	450 MPa, ambient temp ^h , 5 min	Cell culture medium	> 6 (Kingsley et al., 2002a)
<i>Picornaviridae</i>	HAV	400 MPa, ambient temp, 10 min	Cell culture medium	> 2 (Grove et al., 2008)
	HAV	400 MPa, 9°C, 1 min	Oysters	3 (Calci et al., 2005)
	HAV	375 MPa, 21°C, 5 min	Mashed strawberries; sliced green onions	4.3; 4.7 (Kingsley et al., 2005)
	HAV	500 MPa, 4°C, 5 min	Sausages	3.23 (Sharma et al., 2008)
	Poliovirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red (Kingsley et al., 2004)
		600 MPa, 20°C, 60 min		No red (Wilkinson et al., 2001)
	Poliovirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red (Grove et al., 2008)
	Aichivirus	600 MPa, ambient temp, 5 min	Cell culture medium	No red (Kingsley et al., 2004)
	Coxsackievirus B5			No red
Coxsackievirus A9			7.6	

Virus	Inactivation method	Matrix	Log reduction ^a	Reference
<i>Caliciviridae</i>	FCV	275 MPa, ambient temp, 5 min	Cell culture medium	> 6 (Kingsley et al., 2002a)
	FCV	200 MPa, -10°C or 20°C, 4 min	Cell culture medium	5 or 0.3 (Chen et al., 2005)
	FCV	300 MPa, ambient temp, 3 min	Cell culture medium	5 (Grove et al., 2008)
	FCV	500 MPa, 4°C, 5 min	Sausages	2.89 (Sharma et al., 2008)
	MNV-1	400 MPa, 5°C, 5 min	Oyster tissue	4 (Kingsley et al., 2007)
	MNV-1	450 MPa, 20°C, 5 min	Cell culture medium	6.85 (Kingsley et al., 2007)
<i>Leviviridae</i>	MS2	600 MPa, 21°C, 10 min	Cell culture medium	3.5 (Guan et al., 2006)
	MS2	500 MPa, 4°C, 5 min	Sausages	1.47 (Sharma et al., 2008)
Irradiation				
<i>Reoviridae</i>	RoV	2.4 kGy	Oysters; clams	1; 1 (Mallett et al., 1991)
	HAV	UV dose: 40 mW s/cm ²	Lettuce; green onions; strawberries	4.3; 4.2; 1.3 (Fino and Kniel, 2008)
<i>Picornaviridae</i>	HAV	UV dose: 120 mW s/cm ²	Lettuce; green onions; strawberries	4.5; 5.3; 1.8
	HAV	3 kGy	Lettuce; strawberries	1; 1 (Bidawid et al., 2000b)
	HAV	2.0 kGy	Oysters; clams	1; 1 (Mallett et al., 1991)
	HAV	High intensity broad spectrum pulsed light 1 J/cm ²	PBS + 5% FCS ⁱ ; PBS	4.1; > 5.7 (Roberts and Hope, 2003)
	Aichivirus	UV dose: 40 mW s/cm ²	Lettuce; green onions; strawberries	4.0; 2.4; 1.5 (Fino and Kniel, 2008)
	Aichivirus	UV dose: 120 mW s/cm ²	Lettuce; green onions; strawberries	4.4; 3.7; 1.6
<i>Poliovirus</i>	Poliovirus	High intensity broad spectrum pulsed light 1 J/cm ²	PBS + 5% FCS; PBS	3.2; > 6.7 (Roberts and Hope, 2003)
	Coxsackievirus B2	7 kGy	Ground beef	1 (Sullivan et al., 1973)
<i>Caliciviridae</i>	FCV	UV dose: 12 mW s/cm ² ; 200 Gy	Virus suspension with low protein content	3; 1.6 (de Roda Husman et al., 2004)
	CaCV	UV dose: 20 mW s/cm ² ; 200 Gy		3; 2.4
	FCV	UV dose: 40 mW s/cm ²	Lettuce; green onions, strawberries	3.5; 2.5; 1.1 (Fino and Kniel, 2008)
		UV dose: 120 mW s/cm ²	Lettuce; green onions; strawberries	3.8; 3.9; 1.6
<i>Leviviridae</i>	MS2	UV dose: 65 mW s/cm ² , 200 Gy	Virus suspension with low protein content	3; 7 (de Roda Husman et al., 2004)

^aLog reduction represents the reduction in infectivity; ^bRoV: rotavirus; ^cHAV: hepatitis A virus; ^dFCV: feline calicivirus; ^eCaCV: canine calicivirus; ^fMNV-1: murine norovirus 1; ^gNo red: no reduction; ^htemp: temperature; ⁱFCS: fetal calf serum.

Table 4: The efficacy of decontamination procedures on fresh produce to reduce the level of viruses

Virus	Decontamination procedure	Matrix	Log reduction ^a	Reference	
<i>Reoviridae</i>	RoV ^b Water 0.5 min	15 g strawberries/200 ml	1.5	(Butot et al., 2008)	
	NaOCl 200 mg/L 0.5 min		> 1.5 ^f		
<i>Picornaviridae</i>	HAV ^c Water 5 min	10 g lettuce/fennel/carrot/ 100 ml water	0.1; 1; 0.9	(Croci et al., 2002)	
	HAV NaOCl 200 mg/L 0.5 min	15 g strawberries/200 ml	1.0 ^f	(Butot et al., 2008)	
	HAV 20 mg/L chlorine 10 min	1.2 g lettuce/30 ml	> 1.7	(Casteel et al., 2008)	
<i>Caliciviridae</i>	FCV ^d PAA ^e 300 mg/L; 150 mg/L 10 min PAA 300 mg/L; 150 mg/L 10 min Water 10 min	100 g strawberries/100 ml	3 ^f ; 1 ^f	(Gulati et al., 2001)	
		10 g lettuce/100 ml	3 ^f ; 2 ^f		
		100 g strawberries/100 ml; 10 g lettuce/100 ml	2; 2		
	FCV	NaOCl 200 mg/L; 800 mg/L 10 min	100 g strawberries/100 ml	0 ^f ; 1 ^f	(Allwood et al., 2004)
		NaOCl 200 mg/L, 800 mg/L 10 min	10 g lettuce/100 ml	0 ^f ; 1.5 ^f	
		Bleach 50 mg/L, 100 mg/L, 200 mg/L PAA 80 mg/L 3% H ₂ O ₂	3cm ² disks of lettuce in 5 ml sanitizer solution, 2 min	2.2, 2.6, 2.9 2.9 2.8	
		FCV NaOCl 200 mg/L 0.5 min	15 g strawberries/200 ml	> 1.6 ^f	
	FCV NaOCl 300 mg/L 10 min	Cell culture medium	< 2	(Duizer et al., 2004)	
	CaCV ^e NaOCl 300 mg/L 10 min	Cell culture medium	> 3		
	<i>Leviviridae</i>	MS2 Chlorine 100 mg/L 5 min	100 g lettuce /1 l	0.7	(Dawson et al., 2005)
MS2 Chlorine 20 mg/L 10 min		1.2 g lettuce/30 ml	> 1.8	(Casteel et al., 2008)	
MS2 Bleach 50 mg/L, 100 mg/L, 200 mg/L PAA 80 mg/L 3% H ₂ O ₂		3cm ² disks of lettuce in 5 ml sanitizer solution, 2 min	1.9, 2.7, 2.9	(Allwood et al., 2004)	
			2.8		
			2.6		
MS2 10 s H ₂ O ₂ (2%) followed by 30 s UV (0.63 mW s/cm ²), 50°C		5 cm ² sections of lettuce	4.1	(Xie et al., 2008)	
Ca(ClO) ₂ 200 mg/L 3 min	Cut lettuce (5 cm ²)/400 ml	1.7			

^aLog reduction represents the reduction in infectivity; ^bRoV: rotavirus; ^cHAV: hepatitis A virus; ^dFCV: feline calicivirus; ^eCaCV: canine calicivirus; ^fcompared to water; ^gPAA: peroxyacetic acid

4.2.1. Norovirus

4.2.1.1. Acidification

The incubation of Feline Calicivirus and Canine Calicivirus at a pH of 2 or lower for 30 min at 37°C induced more than 5 log inactivation (Duizer et al., 2004). Less than 1 log reduction of MNV-1 was observed when exposed to a pH of 2 at 37°C for 30 min while FCV was reduced by 4.4 log after exposure to the same conditions (Cannon et al., 2006). Infective virus particles were still found when a NoV stool filtrate was subjected to a pH of 2.7 for 3 h (Dolin et al., 1972).

4.2.1.2. Heat treatment

Duizer et al. (2004) observed similar inactivation rates of FCV and CaCV at temperatures ranging from 37°C to 100°C. Also similar thermal inactivation rates at 63°C and 72°C were noted for FCV and MNV-1 (Cannon et al., 2006). Dispersed reductions of FCV for the same time-temperature combination were achieved by (Doultree et al., 1999) and (Buckow et al., 2008). The experimental set-up was likely to be responsible for the differences in heat inactivation rates. MNV-1 showed a reduction of 2.81 log after exposure to 75°C for 0.25 min in 10 g of preheated raspberry puree (Baert et al., 2008b). Slomka and Appleton, (1998) investigated the inactivation of FCV by immersion of cockles in boiling water for 0.5 min and found 1.7 log reduction of FCV. At that time, the internal temperature of the cockles reached approximately 60°C. After 1 min, the internal temperature reached 78°C and FCV [initially 4.5 log TCID₅₀ (50%-tissue culture infectious dose) /g present] could not be detected anymore.

4.2.1.3. High Pressure Processing (HPP)

FCV was reduced by 4 to 5 log at low temperatures (-10°C) when treated with a pressure of 200 MPa (4 min) however the same treatment at 20°C only reduced the titer by 0.3 log (Chen et al., 2005). Kingsley et al., (2007) found only 1.15 log reduction when MNV-1 was treated with a dose of 350 MPa (5 min) in propagation medium at 30°C, while a reduction of 5.56 log was observed at 5°C.

4.2.1.4. Irradiation

UV light treatment of lettuce at a dose of 40 mW s/cm² achieved 3.5 log reduction of FCV (Fino and Kniel, 2008). A 3 log reduction was achieved for FCV and CaCV in tenfold diluted cell culture medium after exposure to UV at a dose of respectively 12 and 20 mW s/cm² (de Roda Husman et al., 2004). Gamma irradiation at a dose of 200 Gy reduced CaCV and FCV respectively by 2.4 and 1.6 log (de Roda Husman et al., 2004).

4.2.1.5. Efficacy of decontamination methods on fresh produce

Removal of viruses by washing depends on produce type. In general, a maximum of 1-2 log removal of micro-organisms could be achieved by washing produce with water (Beuchat, 1998) which is in accordance with the reported decline of viruses.

A treatment of 200 ppm chlorine rendered an additional 1.0 log reduction of MNV-1 present on lettuce compared to washing in tap water (Baert et al., 2009). The application of 200 ppm chlorine to treat strawberries and lettuce did not result in an additional reduction of FCV compared to washing with tap water (Gulati et al., 2001).

High chlorine levels would be required to achieve a 2 to 3 log reduction of viruses on fresh produce. The application of higher concentrations is limited due to sensorial aspects. Prolonging the chlorine treatment would not be useful to increase the efficacy of chlorination since two studies showed that a contact time beyond 10 min made little difference in antiviral activity towards FCV (Duizer et al., 2004; Gulati et al., 2001).

Studies investigating the efficacy of other sanitizers than chlorine upon virus removal are limited. Peroxyacetic acid (PAA) at a concentration of 150 mg/L was tested by (Gulati et al., 2001) to treat strawberries and lettuce, resulting respectively in 1 and 2 log reduction of FCV compared to washing with water. Allwood et al., (2004) showed a comparable decline of MS2 and FCV in the case 200 mg/L chlorine, 3% H₂O₂ and 80 mg/L PAA were used as sanitizers.

Electrolyse oxidizing water (EOW) is a new sanitizer in use in Japan for several years. It is an effective disinfection method, easy to operate, relatively inexpensive and environmentally friendly (Huang, 2008), however, data on virus inactivation using electrolyzed water on food produce is yet unclear.

4.2.1.6. Efficacy of decontamination methods on bivalve shellfish

Depuration rapidly purged out *E. coli* and other bacterial pathogens whereas considerable levels of viral units remained (Schwab et al., 1998; Son and Fleet, 1980). Son and Fleet, (1980) reported acceptable purification after 48 h of depuration with regard to *E. coli*, *Salmonella*, *B. cereus* and *C. perfringens* present in oysters. Depuration of oysters during 48 h reduced *E. coli* by 95% while a minimal decrease (7%) of NoV was established (Schwab et al., 1998). Moreover, human pathogenic viruses were detected at the same frequency in oysters with or without the application of commercial depuration practices in four European countries (Formiga-Cruz et al., 2002).

Specific retention of NoV was observed by (Ueki et al., 2007) observing no decline of NoV genomic copies in artificially contaminated oysters after depuration for 10 days whereas FCV could not be detected anymore after 3 days. It is currently demonstrated that NoV particles bind to glycan ligands, some being very similar to human histo-blood group antigen-like (HBGA) carbohydrates in the digestive tissue of shellfish and may account for the inefficiency of depuration practices (Le Guyader et al., 2006b; Maalouf et al., 2010b).

Besides the type of virus strain, other factors such as the initial contamination level, depuration system, physiological state of the shellfish, seasonal conditions, water temperature and salinity might have an influence on the depuration dynamics of contaminants (De Medici et al., 2001; Dore and Lees, 1995; Kingsley and Richards, 2003; Lees, 2000).

Because shellfish can be held in depuration tanks only for a relatively short period, relaying could be an alternative for heavily polluted shellfish (Lees, 2000). Relaying implies transferring polluted shellfish to natural, pollution free marine environments (Humphrey and Martin, 1993; Son and Fleet, 1980) reported that coliphages were not detected anymore after 2 to 3 weeks of relaying while somatic coliphages were still detected after 5 weeks. RoVLPs (Rotavirus-like particles) could be detected up to 37 days of relaying when an initial concentration of 10⁵ RoVLPs/oyster was present (Loisy et al., 2005).

4.2.2. Hepatitis A virus

4.2.2.1. Acidification

HAV infectious units were present after 5 h exposure to a pH of 1 at room temperature. At 38°C, HAV remained infectious for up to 90 min at pH 1 (Scholz et al., 1989).

4.2.2.2. Heat treatment

One of the most effective treatments to reduce viruses from any food product is to cook the food thoroughly; however this may not be applicable to commodities like shellfish that become unpalatable. Heat treatment to an internal temperature of 85°C - 90°C, maintained for 90 seconds, may destroy viruses in molluscs but careful control is necessary to achieve this without toughening of the shellfish flesh. Hewitt and Greening, (2006) showed differences in HAV inactivation in New Zealand green-shell mussels (*Perna canaliculus*) depending on the method of cooking, where boiling for 3 min was more effective than steaming for 3 min to inactivate HAV. Abad et al., (1997) also showed incomplete

inactivation of HAV and rotavirus after steaming mussels for 3 min after the shells opened. Cooked clams have been implicated in hepatitis outbreaks linked to Peruvian imported bivalves (Pinto et al., 2009; Sanchez et al., 2002). Millard et al., (1987) reported that when the internal temperature of cockle meat was raised to 85-90°C and hold it for 1 min, HAV was inactivated.

Bidawid et al., (2000c) studied heat inactivation of HAV in sterile skimmed milk (0% fat), homogenized milk (3.5% fat) and table cream (18% fat). At 71°C, exposure of 0.16, 0.18 and 0.52 min were needed in respectively skimmed milk, homogenized milk and cream to reduce HAV by 1 log whereas 4 log reduction required 6.55 (skim), 8.31 (homogenized) and 12.67 (cream) min. A longer heat treatment was needed in cream to achieve similar inactivation of HAV compared to milk. The high fat content presumably protected HAV towards heat. However, a recent study found that milk offered no protective effect for HAV (Hewitt et al., 2009). Thermo-resistance of HAV inoculated in synthetic media mimicking chemical characteristics of strawberry mashes was investigated (Deboosere et al., 2004). These experiments showed that high sucrose concentration (indicated as Brix value) increased HAV heat resistance, and that low pH decreased HAV heat resistance. In 1 g strawberry mash (sucrose concentration of 28° Brix, pH 3.8), HAV was lowered by 1 log after a heat treatment consisting of 2 min to reach 85°C followed by 0.96 min at 85°C (Deboosere et al., 2004).

4.2.2.3. HPP

Kingsley et al., (2005) studied the persistence of HAV in mashed raspberries and sliced green onions. HAV exposed to pressures of 375 MPa at 21°C for 5 min was reduced by respectively 4.3 and 4.7 log in strawberry puree and on sliced green onions. Structural and organoleptic changes were observed for treated whole green onions and strawberries, although sliced green onions or strawberry puree might be accepted by consumers and can be used as flavor enhancers or as ingredient for cream, jams, juices or smoothies. HPP was used to treat oysters with a pressure of 400 MPa for 1 min (9.0°C) and induced 3 log reduction of HAV (Calci et al., 2005).

4.2.2.4. Irradiation

UV light treatment of lettuce at a dose of 40 mW s/cm² achieved 4.3 log reduction of HAV (Fino and Kniel, 2008).

Bidawid et al., (2000b) found that 3 kGy was needed in order to achieve 1 log reduction of HAV on lettuce or strawberries. Mallett et al., (1991) reported that 2.0 kGy was able to reduce HAV by 1 log in oysters and clams.

4.2.2.5. Efficacy of decontamination methods on fresh produce

(Casteel et al., 2008) observed at least 1.7 log reductions of HAV on strawberries, tomatoes and lettuce treated with 20 ppm chlorine. However, the actual effect of chlorination is not known in the latter study because the effect of treating inoculated produce solely with water was not mentioned.

4.2.2.6. Efficacy of decontamination methods on bivalve shellfish

(Chironna et al., 2002) reported the presence of HAV genomic copies in 11.1% depurated mussels, marketed in Puglia (South Italy), and 4.4% contained infectious HAV units. Nevertheless, a remarkable decrease in the number of contaminated mussels was observed after depuration.

HAV showed less than 2 log reduction after 4 days depuration of experimentally contaminated mussels while adenovirus and poliovirus were reduced by at least 3 log (Abad et al., 1997; Bosch, 1995).

4.2.3. HEV

Infection of A549 cells was prevented by heating of an HEV-containing cell suspension at 56° C for 30 minutes (Huang et al., 1999). Using HepG2/C3A cells, an HEV genotype 1 strain was nearly completely inactivated at temperatures between 56°C and 60°C for one hour, whereas only about 80%

of a genotype 2 strain was inactivated at 60°C after one hour (Emerson et al., 2005). Time-course analyses showed that about 95% of the genotype 1 strain was inactivated within the first 15 minutes at 56°C although some remaining infectious virus was still detectable after one hour at this temperature. Another study using PLC/PRF/5 cells showed that heating of a genotype 3-containing stool sample at 25°C or at 56°C for 30 minutes did not influence the infectivity whereas heating at 70°C or at 95°C for 10 minutes or at 95°C for 1 minute prevented the growth of the virus (Tanaka et al., 2007). By monitoring of seroconversion of pigs experimentally inoculated with an HEV genotype 3-containing liver suspension it was shown that incubation at 56°C for one hour did not affect infectivity, whereas the suspension was no longer infective after heating at 71°C or at 100°C for five minutes (Feagins et al., 2008). The investigations show that HEV is relative stable against heat treatment and that remarkable differences exist between the different strains; however, heating at 70°C for 10 minutes or at 95°C for 1 minute seems to be sufficient for inactivation of HEV in each case.

4.2.4. Conclusions on effects of treatments used in food processing on viruses

Evidence is given by several studies that depending on the food matrix, viruses can decline during chilling. Yet, persistence of a considerable number of viruses is mostly ascertained during the shelf life period of chilled foods. Viruses can also survive in acidified or dry conditions. The long term survival of viruses in combination with the high infectivity indicates that food preservation methods establishing microbial growth inhibition will not be sufficient to prevent foodborne viral infections.

Preservation methods establishing microbial inactivation such as heating, high hydrostatic pressure processing and irradiation are therefore considered as intervention strategies to reduce the level of viruses. The heat inactivation data obtained in several studies suggested that high temperature, short time pasteurization (e.g. 72°C, 15 s) would accomplish less than 1 log reduction for some enteric viruses and that at least conventional pasteurization (e.g. 63°C – 30 min, 70°C – 2 min) is needed to achieve more than a 3 log reduction. Additionally, the required time-temperature combination depends upon the food matrix and its physical-chemical conditions.

Non-thermal preservation technologies are often preferred to retain nutritional and sensorial aspects of foods e.g. raw bivalve shellfish, lettuce, raspberries and strawberries. High hydrostatic pressure might be able to reduce the level of HAV and NoV by more than 3 log, whereas strains of the genus *Enterovirus* are shown to be very resistant to HPP. Investigation of UV and gamma irradiation to eliminate viruses is limited. More data is required to determine the influence of food matrices. Additionally, the possibility of foodborne viruses to acquire resistance or other mutations needs to be examined.

Decontamination procedures on fresh produce were shown to have a reduction of approximately 1 to 2 log reductions. The efficacy of sanitizers varied between viral strains whereby the explanation for the different rate in decline is difficult to define. A different approach by investigators regarding to the initial virus titer, inoculation procedure and produce/treatment ratio influences the outcome.

Depuration and relaying would be inadequate to remove viruses from live bivalve shellfish within a practical achievable time period. Information regarding the efficacy of relaying shellfish to purge out viral contaminants is scarce but the legal requirement of 2 months for heavily contaminated shellfish seems not to be excessive¹⁵. Alternative purification systems or decontamination technologies are needed to decrease the viral load in bivalve shellfish. For instance, (Tian et al., 2007) suggested the application of HBGA analogs, e.g. pig stomach mucin, in depuration systems to reverse the binding of NoV to oyster tissue.

¹⁵ Council Directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve molluscs. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31991L0492:EN:HTML>

4.2.5. Data gaps on food processing treatments

- Alternative decontamination treatments shown to be of great value to decrease bacterial pathogens should be evaluated with respect to viruses. Especially procedures which can be applied on perishable produce such as raspberries and strawberries are of interest. The possibility of viruses to internalize fresh produce and the effect upon decontamination should be elucidated.
- Inactivation/decontamination studies with regard to viruses are conducted with different viral strains and with different experimental set-ups. In addition, there is a lack of methodology to test NoV infectivity. For these reasons, it is difficult to compare the reduction levels between viruses.

4.3. Diagnostic tests (methods)

4.3.1. Methods of detection for viruses in foods

Analysis of food matrices is complex and many methods have been described (Crocini et al., 2008; Mattison and Bidawid, 2009). The initial contact of the virus with the food may occur at any time during food production, including before the harvest, during processing and at the time of preparation. Almost any kind of food can be involved in virus transmission, but as presented above a limited number of foods are most commonly associated with outbreaks and thus are the target of developed methods. One of the first challenges to analyze food contamination is the sampling strategy to choose representative samples (Pinto and Bosch, 2008). The second important step is the method sensitivity as the level of contamination is expected to be low. Virus particles are very often on the food surface and different factors inherent to food surface or virus specificity may interfere (Le Guyader and Atmar, 2008). The knowledge of these binding mechanisms is helpful to improve recovery from food matrices in the first step of methods (Mattison and Bidawid, 2009). Elution, using basic buffer or chemical treatment are often used before concentration step based on filtration or precipitation (Table 5).

Table 5: Example of methods used to recover viral particle form representative food.

Matrices	Elution	Concentration	Ref
Lettuce	Glycine buffer pH 8.8	Ultrafiltration	(Cliver et al., 1983)
Lettuce	Phosphate buffer pH 7.5, filtration	addition of glycine buffer/ 3% beef extract, filtration	(Dubois et al., 2006)
Green onions	Sodium bicarbonate	Ultracentrifugation	(Kurdziel et al., 2001)
Green onions	Tryptose phosphate, 6% glycine pH 9.5	PEG 8000 precipitation	(Guevremont et al., 2006)
Berries	Glycine buffer, Tris/1% beef extract pectinase	Centrifugal filter 100K NMWL	(Butot et al., 2007b)
Berries	Glycine buffer, Tris-HCl, 3% beef extract pH 9.5, pectinase	PEG precipitation, chloroform-butanol, PEG precipitation	(Baert et al., 2008a)
Pasta	Trizol		(Baert et al., 2008a)
Ready to eat produce	3% beef extract solution pH 8.5	PEG 6000 precipitation	(Allwood et al., 2004)
Delicatessen	PBS and freon, centrifugation	PEG 6000 precipitation	(Schwab et al., 2000)
Rolled cabbage and macaroni	PBS and freon, centrifugation	antibody coated on beads	(Kobayashi et al., 2004)
Ready to eat food	Citrate buffer	HBGA fixed on magnetic beads	(Morton et al., 2009)

4.3.2. Standardisation of methods for detection of NoV and HAV viruses in foods

A major factor limiting the uptake of virus testing into regulatory food controls world-wide is the current absence of any standardised and validated methods. In 2004 the European Committee of Standardisation (CEN) initiated the development of a standard method for detection of norovirus and hepatitis A virus in foodstuffs based on PCR (Lees, 2000). The standard developed by a working group of expert European laboratories is now well advanced and due for publication in 2012 (Lees and CW, 2010).

Food samples present a challenging matrix and the standard method needed to be capable of extracting low levels of contaminating virus and presenting them in a non-inhibitory extract to a sensitive PCR assay. Key aspects of the developing method were tested by inter-laboratory evaluations to ensure

robust performance. The method targets the at risk food matrices of molluscan shellfish, soft fruits, salad vegetables, bottled water and food surfaces (both the surfaces of foods and food preparation surfaces). For bivalve molluscs dissected digestive diverticulum (digestive gland) is used as the starting material with further enzymatic digestion using proteinase K (Jothikumar et al., 2005). For food surfaces swabbing is employed followed by elution into sample buffer (Scherer et al., 2009). For both soft fruit and salad vegetables viruses are eluted with agitation followed by recovery using PEG (polyethylene glycol)/NaCl precipitation (Dubois et al., 2007). For bottled water viruses are adsorbed to a positively charged membrane, eluted and then concentrated by ultrafiltration (Butot et al., 2007a). Following initial sample treatment all food matrices are then further processed by a common nucleic acid purification and PCR platform.

Nucleic acid purification utilises guanidine thiocyanate (GITC) to denature viral coat proteins in combination with magnetic silica particles to bind released nucleic acid, which is then purified through successive washing stages before final elution in a small volume. Reverse transcription and PCR utilises a one-step approach using specific primers in order to simplify the procedure as much as possible. However, commercial one-step kits must utilise enzymes specifically engineered for use with low abundance targets. TaqMan PCR real-time chemistries are stipulated for the amplification since: the closed tube format is less susceptible to contamination; is logistically efficient; incorporates a probe based confirmation step; is quantitative; and is more amenable to standardisation than conventional PCR.

To maximise sensitivity real-time PCR assays are run separately for NoV genogroup I, NoV genogroup II, and HAV. Cross-reactive real-time PCR primers and probes are directed in the ORF1-ORF2 junction region for NoV (Le Guyader et al., 2009; Svraka et al., 2007) and in the highly conserved 5' non-coding region for HAV (Costafreda et al., 2006). Exact primer/probe configuration within these regions is flexible to accommodate potential future strain variability. However the standard requires the use of peer reviewed primers/probes shown to be sufficiently sensitive and cross-reactive. The standard includes an informative annex with recommended primers/probes suitable for detection of all current strains of human NoV and HAV.

The method is highly sensitive in order to detect the low levels of virus found in environmentally contaminated samples and hence also vulnerable to both cross-contamination (false positives) and potential matrix interferences (false negatives). Thus a comprehensive suite of controls was also developed to cover: positive and negative process controls; negative RNA extraction control; positive RT-PCR and RT-PCR inhibition controls; negative and positive PCR controls. The positive process control measures the recovery of virus during the whole extraction and test procedure using a heterologous non-enveloped positive-sense ssRNA virus spiked into the test sample and assayed in parallel with the target viruses. During the development of the method inter-laboratory studies by the working group successfully utilised the MC0 strain of Mengo virus (Costafreda et al., 2006) as a process control. The negative process control is a known negative sample that is taken through the entire extraction procedure and analysed. The RT-PCR inhibition control checks for potential matrix suppression by comparison of amplification of an external RNA template added to test material and a control well. Taken together the controls generate data on all aspects of the assay and are utilised to determine the acceptability of test performance against established quality control criteria.

The standard incorporates two parts covering both quantitative and qualitative detection. The differences principally relate to the necessary suite of controls and the calibration curves required for determining virus template concentrations. Quantitation is based on a plasmid DNA calibration curve for each assay (NoV GI, NoV GII, HAV) with plasmid DNA concentration measured using spectrometry at 260nm. Results are reported in the standardised form of detectable virus genome copies per gram of material tested. Qualitative assays will report presence or absence with reference to their limit of detection. Formal validation studies are planned to characterise the method according to the international requirements.

4.3.3. Detection of HEV in meat and meat products, and pigs.

No standardized methods are available for detection of HEV in meat and meat products. Although some reports on successful isolation and propagation of HEV in tissue culture exist, all of the described tissue culture systems are limited as they are inefficient and rely on high inoculation titres (Chandra et al., 2008). Therefore, molecular methods are preferred for HEV detection in food. In order to assess the performance quality of the applied protocol, control reactions as described in section 4.3.2 should be carried out along with each analysis.

Several techniques are established for the extraction of viral RNA from muscle tissue samples, which can be readily used for meat and liver tissue analysis; methods for analysis of further processed meat products have not been sufficiently evaluated so far. Generally, the tissue is first chopped and homogenized, thereafter lysed and the RNA is purified from the lysate in a last step. Many protocols use chaotropic salts such as guanidine isothiocyanate for tissue lysis. The RNA can thereafter be purified by either using phenol/chloroform extraction or silica-based purification methods. Many commercially available kits exist for the isolation of RNA from tissue samples. The isolated RNA is thereafter analysed by PCR; several protocols for conventional (Huang et al., 2002; Preiss et al., 2006; Schlauder et al., 1999) or real-time RT-PCR (Bouwknegt et al., 2009; Gyarmati et al., 2007; Jothikumar et al., 2006) for the detection of HEV genotypes 1 to 4 in humans and animals have been published.

Some of the available ELISA kits can detect anti-HEV immunoglobulins independently from the analyzed species thus also enabling testing of pigs and other animal species. In other cases, antigens from human assays have been used in combinations with species-specific secondary antibodies for serological testing of animal species. Generally, a high divergence of results has been observed for identical pig serum samples by using different serological assays (Bachlein and Grummer, 2010).

4.3.4. Detection of viruses in humans

Various reverse transcription-PCR (RT-PCR) assays have been designed to detect NoV in clinical samples such as faecal samples or vomit, and also in environmental samples, such as surface swabs, or food and water. The target genomic regions have not been standardised, with the exception of the work done by the CEN group (Marshall and Bruggink, 2006). RT-PCR is relatively sensitive, offering the possibility of detecting a low quantity of virus, using degenerate primers targeting conserved genomic regions. However, due to the high degree of variation among NoV, some NoV strains may not be detected. In clinical practice this usually is not problematic given the dominance of a limited number of genotypes. In other situations and in reference laboratories care should be taken to monitor test-performance against less common genotypes. Real-time PCR is increasingly used, which is more sensitive and faster than RT-PCR. Using real-time PCR with virus-specific-primer and probe combinations in multiplex assays, the detection of multiple different viruses in one test has become feasible. Additionally, real-time assays are semi-quantitative, i.e., a decrease in Ct values for the same virus indicates that the amount of viral RNA present in samples has increased, which may be indicative for clinical significance of the test results. This enables the use of viral loads as parameter in interpretation of test results. Although this is not yet common practice, the approach holds some promise for the future (Phillips et al., 2009). NoV may also be shed by asymptomatic individuals, and on average lower viral loads have been found in such patients. Also, viral loads may differ between genotypes, and this may have consequences for the probability of subsequent onward transmission (Chan et al., 2006).

Enzyme immunoassay (EIA) tests have been developed for the detection of NoV antigen in stool samples, and several of these tests are commercially available. Advantages of EIA testing over PCR based assays include simplicity (no specialized equipment or skilled personnel required) and speed (rapid bed-side tests have been developed based on an EIA that promise results within 15 minutes). The EIAs use either monoclonal or polyclonal antibodies specific for a limited number of antigenically-distinct NoV genotypes, which can be problematic in the detection of antigenic variants or emerging genotypes (Gray et al., 2007). Knowledge of the local circulating NoV genotypes is

helpful in evaluating the efficiency of the EIA in a particular setting (de Bruin et al., 2006). Moreover, if outbreak samples are negative by the EIA test, they should be further screened by RT-PCR. The low sensitivity of EIA tests (between 44 and 59%) makes them less suitable for diagnosing sporadic cases, unless negative results are, again, followed by RT-PCR analysis. Currently serology has no role in the diagnosis of NoV infections.

In contrast, for hepatitis A and hepatitis E infection, diagnosis is done primarily on the basis of antibody detection assays. Commercial assays are available and are used routinely for HAV, and to a lesser extent for HEV. Viral RNA can be detected by RT-PCR in serum and in stool samples in patients with acute HAV or HEV infection, but this is not used routinely in most clinical laboratories. Given the low prevalence of HEV, the rate of false positive serological assays is relatively high, and confirmation of reactivity by immunoblot and RNA detection by RT-PCR are recommended (Herremans et al., 2007). There is some evidence that diagnosis of the genotype 3 HEV infections by commercial assays developed for diagnosis of HEV in travellers (hence mostly infections with genotypes 1 and 2) is less sensitive. In addition, in immunocompromised patients, prolonged viremia has been detected, sometimes in the absence of a measurable antibody titre (Haagsma et al., 2008). Although these tests for diagnosis of human hepatitis E are broadly available, testing is currently only rarely done, probably due to the fact that hepatitis E is still considered as an exotic disease by the majority of general practitioners.

4.3.5. Molecular typing, including new developments for source attribution

Since the mid 1990s, defining NoV genotypes has been done on the basis of complete capsid gene (ORF 2) sequencing, where a new genotype was defined when strains differed by more than 20% (Green et al., 2000; Vinje et al., 2000) at amino acid level from their nearest neighbour. With the rapid accumulation of more sequence data, this absolute distinction between genotypes became less obvious, and genotyping is now done based on phylogenetic clustering (Zheng et al., 2006). Standardisation of nomenclature has been agreed (Duizer et al., 2008), with a web-based typing service as core facility (<http://www.rivm.nl/mpf/norovirus/typingtool>). At present, 8 GGI and 21 GGII genotypes have been identified, respectively.

Many laboratories perform sequencing of NoV positive samples. Determining the genotype and possible signature mutations enables linking of patients or outbreaks, or finding a common source of infection. Again, several genomic regions (A to E) can be analyzed and there is no international standardization of this approach (Figure 3). For surveillance purposes these partial genomic sequences are used to monitor trends, whereas the highly variable P2 domain of the capsid is sequenced for addressing questions regarding transmission routes, e.g., in assessing hospital epidemiology, but also for linking patients to a source (Xerry et al., 2008). Ideally, virus typing in positive food extracts and patients should be done with agreed standardized targets, but the challenges in identifying viral RNA in foods is a limiting factor.

Genotyping has been found to be meaningful for understanding NoV epidemiology as viruses belonging to different genotypes differ in their epidemiological behaviour (Gallimore et al., 2004b; Kroneman et al., 2008). In outbreak surveillance, GGII4 viruses are by far the most commonly identified viruses, often associated with outbreaks in healthcare settings and with person-to-person transmission. The non GII4 viruses are more often found in other settings, including those where food has been implicated as the source of an outbreak (Kroneman et al., 2008). This information can be used to triage the outbreaks reported to public health officials: in some regions, particularly during the winter season, outbreaks are so common that it is impossible to follow-up on each of them (Verhoef et al., 2009). Further support for unravelling the source of outbreaks may come from careful comparison the diversity of viruses identified in routinely sampled foods (e.g. shellfish) with that of viruses found in humans, an approach similar to what has been used for many years for assessing the role of different animal species in human Salmonellosis. A first application was recently published, indicating this might indeed be used. However, it requires systematic routine data collection and integration into a common database, a practice that is not routine in Europe (Koopmans et al., 2003).

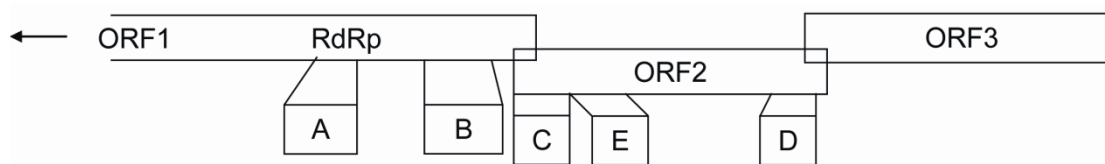


Figure 3: Schematic representation of the locations of the genomic regions of NoV used for genotyping. Adapted from (Siebenga et al., 2009; Vinje et al., 2004)

Hepatitis A genotyping has not been used very extensively, although the first studies defining lineages of HAV date from over 20 years ago. Robertson et al., (1991) used sequence diversity in the VP1-2a junction. In this region, seven genotypes were initially defined, whose genetic distance was >15% nucleotide variation. After refining this classification through the addition of more sequences, only six genotypes exist at the present time (Costa-Mattioli et al., 2002; Lu et al., 2004). Three out of these six genotypes (I, II and III) are of human origin while the others (IV, V and VI) are of simian origin. Genotypes I and II contain subgenotypes (Ia, Ib, IIa and IIb) defined by a nucleotide divergence of 7-7.5%. The use of long genomic regions (Costa-Mattioli et al., 2002) has recently been recommended for a broad molecular typing of HAV, but this is far from common practice due to lack of resources.

As can be seen above, the genome of HAV is far less diverse than that of noroviruses, and only a single serotype has been identified. The VP1-2A target has been used quite extensively for genotyping, but provides relatively poor resolution. Most promising seems to be the VP1 region, and specifically the N-terminal part, although in 1 study 1100 nucleotides in this area were sequenced and 100% homology was found comparing the outbreak strain to some 'background' sequences (Dentinger et al., 2001). Also the 2C area appears to be relatively variable, although only few strains have yet been sequenced in this area (Joshi et al., 2008). More in general, a larger area sequenced gives better results. Genetic sequencing may be used to confirm a foodborne HAV outbreak, but the methodology to be used depends on the nature of the source and the level of endemicity of HAV in the region. In addition, the robustness and level of resolution of the genotyping result in part depends on the choice of the targets that were used. This should be taken into consideration when using sequence data as evidence. There is currently no example of the systematic use of sequence data from notified HAV cases to link cases with unknown risk factors to food-sources. Examples from outbreak investigations suggest that this may be possible.

Molecular typing may also be used to support source tracing of HEV infections, but this is even less standardised and not used routinely. Genotyping of HEV is performed by sequencing of PCR products targeting ORF1 or ORF2 using several PCR protocols as mentioned in Section 4.3.3 and subsequent comparison of the sequence with known strains. Because of the distinct geographical distribution of genotypes 1, 2 and 4 (see Section 2.2.2), genotyping may give a first indication on the global region, in which the infection took place. Further classification into subtypes is possible (Lu et al., 2006), which may enable a further narrowing of the possible origin of infection and which is especially useful for the extremely diverse genotypes 3 and 4. In order to confirm foodborne and/or zoonotic transmission of HEV in a certain case, sequences derived from food and/or animal has to be compared directly with that derived from the patient.

4.4. Occurrence data (NoV, HAV and HEV) in food

4.4.1. Occurrence in shellfish

When viruses are present in shellfish they often occur in low numbers, as compared to clinical samples. Nevertheless, they are present in sufficient quantities to pose a health risk. This low level of contamination has made it necessary to develop highly sensitive viral extraction methods to ensure

virus recovery from shellfish tissues. The observation that viruses are concentrated in digestive diverticulum tissues led to the development of a method that represented a major step in the improvement of extraction methodologies (Atmar et al., 1995; Metcalf et al., 1980). Focusing the analysis of shellfish on the digestive tissues, which represent about one tenth of the total animal weight for oysters and mussels, enhances assay performance by eliminating tissues (i.e. adductor muscle) that are rich in inhibitors (Atmar et al., 1995). With the exception of small species, such as clams or cockles, in which dissection may be technically difficult, most of recent methods are based on dissected tissues.

Factors explaining the observed variability reported in Table 6 include the analysis of shellfish collected in different years, the use of different concentration/extraction methods, and the use of different RT-PCR assays. It is also possible that prevalence surveys with positive findings may be over-represented based upon publication bias. To limit data variation only papers published later than 2000 were considered as different improvements both in shellfish method extraction and RT-PCR protocols were achieved. Also, only papers presenting data from more than 25 samples were considered, either from imported shellfish or collected locally. Data are presented in commercial (class A or B, see Table 10) and non commercial area as these shellfish samples may have been collected only for scientific research and are not for human consumption. Reported prevalence of NoV detection varies from 0% to 79% in commercially distributed shellfish and for hepatitis A virus from 0 to 43% (Table 6). In non commercial area, the same range of variations are observed: NoV were detected from 0 to 60% and hepatitis A virus from 0 to 49%.

HEV has also been found in shellfish, although systematic studies are lacking. The presence of HEV of genotype 3 has been reported for 2 of 32 packages of Yamato-Shijimi in Japan (Li et al., 2007).

Table 6: Reports of human enteric viruses (NoV, HAV) in shellfish collected in different countries.

Shellfish	Country	Number samples analyzed	Commercial area		Non commercial area		Nucleic Acid detection		Reference
			HAV	NoV	HAV	NoV	Method		
Oyster	France	181	0	23	8	25	RT-PCR & hyb	(Le Guyader et al., 2000)	
	Imported	87	0	9			RT-PCR & seq	(Beuret et al., 2003)	
	Japan	191		9			rRT-PCR	(Nishida et al., 2003)	
	Imported	507		10			RT-PCR & seq	(Cheng et al., 2005)	
	Japan	41				60	RT-nested PCR	(Ueki et al., 2005)	
	Netherlands	66	0	0	0	0	RT-PCR	(Lodder-Verschoor et al., 2005)	
	Brazil	57			49		RT-PCR & hyb	(Sincero et al., 2006)	
	US, West coast	16		43			RT-PCR & hyb	(Costantini et al., 2006)	
	East coast,	16		6					
	Gulf coast	9		0					
	Japan	1512		5			rRT-PCR	(Nishida et al., 2007)	
	India	100	0	0			RT-nested PCR	(Umesha et al., 2008)	
	UK site 1	145		52			rRT-PCR	(Lowther et al., 2008)	
	site 2	92		79					
	Irland	167		31*		25*	rRT-PCR	(Flannery et al., 2009)	
	US Gulf,	174	6	3			rRT-PCR	(Depaola et al., 2010)	
	Mid-Atlantic	100	2	3					
North-Atlantic	53	4	7						
Pacific	60	3	5						
Mussels	Italy	36	36				RT-PCR	(Crocchi et al., 2000)	
	Italy	89	34				RT-nested PCR	(De Medici et al., 2001)	
	Sweden	54			0	20	RT-nested PCR	(Hernroth et al., 2002)	
	Sweden	54	0	28	0	26	RT-nested PCR	(Formiga-Cruz et al., 2002)	
	Italy	209	23		20		RT-nested PCR	(Chironna et al., 2002)	
	Spain	54				20	RT-nested PCR	(Muniain-Mujika et al., 2003)	
	Norway	681		7			RT-nested PCR	(Myrmel et al., 2004)	
	Tunisia	23			26	35	RT-PCR & hyb	(Elamri et al., 2006)	
Mussels & oysters	Greece	144	22	6	11	23	RT-nested PCR	(Formiga-Cruz et al., 2002)	
	Spain	104	4	26	0	35			
	UK	173	0	32	3	40			
	Netherlands	41		16			RT-nested PCR	(Boxman et al., 2006)	
Clams, mussels & oysters	Italy	129	12		18		RT-PCR & seq	(Macaluso et al., 2006)	
	Italy	137		2		0	RT-PCR	(Gabrieli et al., 2007)	
	Italy	120	0	0	0	8	RT-boosterPCR	(Suffredini et al., 2008)	
	Italy	116		12*			RT-PCR & seq	(Terio et al., 2010)	
Clams, cockles & mussels	Spain	41	0	46	0	17	rRT-PCR	(Vilarino et al., 2009)	
	Spain	160	43		44		rRT-PCR	(Manoso et al., 2010)	
Clams	Spain	15			53		RT-nested PCR	(Sunen et al., 2004)	
	India	74	0	0			RT-nested PCR	(Umesha et al., 2008)	
Cockles	Italy	53	36				RT-nested PCR	(De Medici et al., 2001)	

Number represent % of samples positive for the designated enteric virus; blanks were used when samples were not evaluated for the designated virus. *: only NoV GII searched.

Abbreviations: HAV = hepatitis A virus; NoV = norovirus, hyb: hybridization, seq: sequencing, rRT-PCR: real-time RT-PCR
Commercial area: class A or B regarding EC regulation, non-commercial area: class C or forbidden area (as written in the paper)

4.4.2. Occurrence in fresh produce

Hitherto, robust analytical methods suitable for routine analysis of fresh produce for contamination with foodborne viruses have been unavailable, and consequently there is no extensive information regarding virus numbers in contaminated foodstuffs. However, some of the information which can be gleaned from outbreak investigations hints at the scale of contamination of fresh produce when it occurs. In several outbreaks, hundreds of people have become ill, and often this has occurred in different locations, e.g. the outbreak of gastroenteritis in Denmark in 2005 (Falkenhorst et al., 2005), which took place in two Danish cities, and the outbreak of hepatitis in the USA in 1997, in which several states were involved. This strongly indicates widespread contamination of a large amount of the original foodstuff batch, especially since viruses cannot replicate in foods. Another indication of the potential scale of contamination is given in the information acquired during the outbreak of hepatitis which occurred in Scotland in 1983 (Reid and Robinson, 1987). Here, a 3 lb (~1.5 kg) batch of raspberries was implicated as the vehicle of transmission. One of the people who became ill was a caterer who prepared a dessert from the fruit, and reported that they had merely tasted it, probably by dipping the edge of a spoon into the pureed fruit and touching it to their tongue. If the tiny quantity of contaminated food that was consumed in that action contained a dose of HAV (>10 particles), the quantity of HAV in the contaminated raspberry batch may have been massive.

While there is some information on foodborne viral infections caused by consumption of contaminated fresh produce, and also on detection of virus in fresh produce implicated in outbreaks, there is little information on the general occurrence (prevalence) of viruses in different fruits and vegetables. This is because there is no routine or regular monitoring of fresh produce for the presence of viral contaminants. Some recent information (from an EFSA call for data) has been made available from some EU Member States, revealing that NoV has been detected in contaminated produce, for example in fresh salad samples in Austria in, and in berries in Finland. A survey of salad vegetables conducted in the Slovak Republic in spring 2008 found that 5 out of sixty samples were contaminated with NoV; these samples were of lettuce, leeks, spring onions and mixed vegetable, and all were collected in large retail stores.

In Canada, between April and November 2009, 328 samples of packaged leafy greens were evaluated for the presence of NoV and rotavirus (Mattison et al., 2009). In total, 275 samples showed recovery of the process control and were considered valid for further analysis. Of these 275 samples, 148 (54%) were positive for NoV and 1 (0.4%) for RoV group A by RT-PCR. Sequencing was possible in 16 of the 148 positive NoV leafy greens and 1 of the 1 RoV positive leafy greens. Most NoV detected belonged to NoV genogroup I. Neither associated illness complaints nor outbreaks were reported.

In Belgium, a survey of 75 fruit products (30 soft red fruits, 30 cherry tomatoes and 15 fruits salads) for the presence of NoV was performed in April-May 2009 (Stals et al., 2011). According to the recovery of the process control, 29 soft red fruits, 8 cherry tomatoes and 2 fruits salads were valid for further analysis towards NoV. Ten of the 29 (34.5%) soft red fruits, 7 (87.5%) cherry tomatoes and 1 (50%) fruit salad were found positive by real-time RT-PCR. However, sequencing of the positive signals obtained by real-time RT-PCR failed. This can be explained by the detection of NoV in the tested fruit products close to the detection/quantification limit and the lower sensitivity of conventional RT-PCR which was carried to obtain an amplicon useful for sequencing. Neither associated illness complaints nor outbreaks were reported.

4.4.3. Occurrence in animal products (meat)

HEV has been detected repeatedly in meat from pigs, wild boar and, to a lower extent, in deer. Detection of HEV in wild boar meat directly linked to human hepatitis E cases in Japan is described in section 2.2.2. The prevalence of HEV RNA in livers of hunted wild boars as assessed by several studies in Europe and Japan ranges from 3 to 25 % (Pavio et al., 2010). Commercially sold porcine livers have been found to contain HEV RNA, with detection rates of 1.9 % in Japan, 6.5 % in the The Netherlands, 10.8 % in Korea, 0.83 % in India and 11 % in the USA (Pavio et al., 2010). Presence of infectious HEV could not be confirmed for Dutch commercial porcine livers (Bouwknegt et al., 2007),

whereas those obtained in the USA contained infectious HEV (Feagins et al., 2007). Twenty of 39 muscle samples from pigs of an HEV infection experiment, which may serve as proxies for pork meat at retail, contained HEV RNA (Bouwknegt et al., 2009).

No systematic data on the occurrence of HEV in meat or meat products exist except for these sporadic studies. In addition, no data on the occurrence of HEV in food other than pig and wild boar meat are available.

4.4.4. Occurrence in food handling environments

NoVs are transmitted primarily through the faecal-oral route, either directly from person-to-person via contaminated hands, or indirectly via contaminated food or water or contact with contaminated surfaces. A survey with respect to risk factors to contract NoV gastroenteritis, showed that having a household member with gastroenteritis, contact with a person with gastroenteritis outside the household, and poor food-handling hygiene were associated with illness (de Wit et al., 2003). Foodborne NoV outbreaks are often linked to food handlers who infect foods that are eaten raw or not further processed (ready to eat (RTE) foods) prior to consumption (Baert et al., 2008a). In many of these outbreaks, a sick food-handler or food-handler with a recent history of gastroenteritis was noticed (Anderson et al., 2001; Boxman et al., 2007; de Wit et al., 2007; Godoy et al., 2005; Lederer et al., 2005; Payne et al., 2006; Sakon et al., 2005; Schmid et al., 2007). Workers have often been in contact with ill family members including children before the worker handled food. An example is a foodborne NoV outbreak which occurred after a pre-Christmas celebration among a group of local foresters in Austria in December 2007 where 21 out of 63 persons became ill (Kuo et al., 2009). Ham roll remained significantly associated with disease risk and was most likely contaminated with NoV during preparation by a disease-free kitchen assistant, whose infant became sick with laboratory-confirmed NoV gastroenteritis 2 days before the party. Outbreaks of infection with hepatitis A virus associated with a foodhandler have also been reported (Chironna et al., 2004).

Food handlers can contaminate food either with particles from vomit (NoV) or from faeces (NoV/HAV) when practicing insufficient personal hygiene especially when shedding viruses themselves, e.g., after using toilets, but also after taking care of infected persons (e.g., changing of diapers) or cleaning toilet areas used by infected persons (Codex committee on food hygiene to the control of viruses in food)¹⁶.

NoV, *Salmonella* and HAV are among the most common agents in foodborne outbreaks where food workers are responsible for the outbreak (Greig et al., 2007). The most common food worker errors identified in relation to outbreak of NoV and HAV are food handling by an infected person or carrier of virus together with bare-hand contact by handler or worker or preparer (e.g., with RTE foods) and failure to properly wash hands when necessary (Todd et al., 2007). Poor personal hygiene was also identified as a contributing factor in outbreaks with NoV assigned as the causative agent (Noda et al., 2008).

Asymptomatic food workers are implicated more frequently than symptomatic workers, which helps explain the difficulty in detecting and stopping an outbreak by excluding ill food workers (Todd et al., 2007). Ozawa et al., (2007) showed that many asymptomatic food handlers tested positive for NoV GII.4 strain in Japan. The number of virus shed by symptomatic and asymptomatic food handlers was similar, indicating the potential hazard of these highly contagious viruses.

In addition, more patients tend to be involved in outbreaks due to food handlers compared to oyster related outbreaks because mostly large food serving facilities are implicated compared to oyster associated outbreaks (Rizzo et al., 2007).

¹⁶ www.codexalimentarius.net/download/report/753/REP11_FHREVe.pdf

Food handlers can also contaminate food when transferring viruses from contaminated surfaces to hands during preparation of ready-to-eat food or when transferring viruses from contaminated food to other ready-to-eat foods. Inanimate surfaces include contaminated utensils, e.g., chopping equipment, such as a dicer; cutting knives, and serving utensils. A gastroenteritis outbreak in a restaurant was reported and swabbing of the hands of staff members preparing the food, toilet seats and the grip of the knife used to cut the bread demonstrated the presence of NoV RNA (Boxman et al., 2009). The sequence was identical to the clinical samples providing evidence for the spread of NoV by food handlers and food contact surfaces. In a systematic survey of food establishments throughout the course of one year, in total, 42 (1.7%) out of 2496 environmental swabs from 35 (4.2%) catering companies tested positive. In contrast, NoV was detected in 147 (39.7%) of the 370 samples for 44 (61.1%) of the 72 establishments associated with outbreaks of gastroenteritis (Boxman et al., 2011).

In addition, transfer of HAV from artificially contaminated fingerpads of adult volunteers to pieces of fresh lettuce was observed by (Bidawid et al., 2000b). Touching the lettuce with artificially contaminated fingerpads for 10 s resulted in transfer of 9.2 % +/- 0.9 % of the infectious virus. Nearly 46 +/- 20.3, 18 +/- 5.7, and 13 +/- 3.6 % of infectious virus was transferred from contaminated fingerpads to ham, lettuce, and metal disks, respectively (Bidawid et al., 2004).

The occurrence of NoV and HAV in the food handling environment is clear mainly from outbreaks where the foodhandler was pointed out as the origin of outbreaks and from studies showing the ability of viruses to be transferred from hands to food. These findings provide direct evidence for the feasibility of transmission of norovirus by a food handler to food. Education of food handlers on the infectivity of norovirus and updating of hygienic codes are strongly recommended.

4.5. Food consumption data (bivalve molluscs and berries)

Data has been extracted from the EFSA's comprehensive consumption database regarding berries and small fruits, water molluscs and crustaceans. Information on the figures and surveys carried out at national level by a number of EU MS are provided on tables 11, 12, and 13 (Appendix A). From the data presented it is evident that there is a large variation in consumer habits across different member states, both in the percentage of the consumer sample that declared consumption of the specific food item, and in the average daily amount of product consumed. This would make the task of a EU risk assessment very complex, as the consumption figures are very different depending on the member state.

5. Risk Characterisation

5.1. Review of RA activities for FBV

Some risk profiles, the first part of a RA, have been reported on FBV, such as a risk profile of Norwalk-like virus in molluscs (raw) in New Zealand (Greening et al., 2003)¹⁷, on foodborne NoV infections¹⁸ and for HEV (Bouwknegt et al., 2009). Due to data limitations, it was concluded at an international meeting of experts that undertaking a full quantitative risk assessment for FBV may be premature (FAO/WHO meeting report¹⁹).

Few full quantitative viral risk assessments for food have been published to date (Hamilton et al., 2006; Mokhtari and Jaykus, 2009; Munoz et al., 2010; Petterson and Ashbolt, 2001; Petterson et al., 2001; Pinto et al., 2009; Stine et al., 2005a). These studies either refer to NoV, HAV or viruses in general and regard crops, shellfish or retail food.

¹⁷ <http://www.nzfsa.govt.nz/science/risk-profiles/norwalk-like-virus-in-raw-mollusca.pdf>

¹⁸ HPA 2004 Final Scientific Report 184-1-318 Microbiological Risk Assessment for Norovirus infection-Contribution to the overall burden afforded by foodborne infections.

¹⁹ Viruses in Food: Scientific Advice to Support Risk Management Activities. Meeting Report Microbiological Risk Assessment Series, No. 13, 2009;
<http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=15&codcch=751>

Four models focus on the use of irrigation water for salad crops (Hamilton et al., 2006; Petterson and Ashbolt, 2001; Petterson et al., 2001; Stine et al., 2005a). It was concluded that the risk of infection was found to be variable depending on type of crop, irrigation method, and days between last irrigation event and harvest. Moreover, predicted infection rates were significantly underestimated if the presence of a persistent sub-population of viruses was not considered in the decay kinetics of the risk model. Petterson et al., (2001) modelled the clinging of viruses to lettuce crops through sprayed irrigation water best fit by a negative binomial distribution. However, the volume of retained water was obtained from a study in which lettuce heads were completely immersed in water (Shuval et al., 1997). This procedure may not represent the volume of water retained after irrigation, which therefore should be examined. Furthermore, actual consumption data were not included in the model, but instead the risk was verified for a fixed consumption of 100 g. Stine et al., (2005a) estimated the maximum concentration of HAV on crops resulting in a 1:10,000 annual infection risk. Hamilton et al., (2006) estimated the risk of infection for enterovirus due to consumption of arable crops, including lettuces, that were contaminated by irrigation water. The authors conducted field experiments to estimate the amount of irrigation water retained on broccoli and cabbage, and used the previously described estimates from Shuval et al., (1997) for lettuce. The remainder of the study is similar to that of Petterson et al., (2001), with the difference that consumption is represented as function of bodyweight by Hamilton et al., (2006). Up to date mostly worst case scenarios are modelled, however, practices such as subsurface, furrow, or drip irrigation and postharvest washing/disinfection and food preparation could substantially lower risks and need to be considered in future models (Hamilton et al., 2006). The risk from use of virus contaminated treated wastewater for irrigation of crops was assessed by Munoz et al., (2010). The assessment of risks from viruses showed a very low probability of infection.

A quantitative exposure model for the transmission of norovirus in retail food preparation was published by Mokhtari and Jaykus, (2009). This mathematical approach to modeling the transmission of gastrointestinal viruses should facilitate comparison of potential mitigations aimed at reducing the transmission of foodborne viruses.

Pinto et al., (2009) performed a risk assessment on shellfish-borne outbreaks of hepatitis A. The estimated risk of infection after consumption of lightly cooked clams matched actual epidemiological attack rates.

With respect to the models used, Regli et al., (1991) and Haas et al., (1993) provide a theoretical background to assess an infection risk due to consumption of drinking water, which was used for FBV. Regli et al., (1991) describe the assumptions that are required to be made in virological risk assessment and evaluates different dose-response models (i.e., exponential vs. beta Poisson). Haas et al., (1993) provides an approach for including uncertainty and variability into risk assessments. The theories about distributions, homogenization, dose-response models and uncertainty and variability described in these two papers are valuable for QMRA's for FBV in future.

Table 7: Quantitative Risk Assessments for FBV

Matrix	Virus	Title publication	Reference
Salad crops	Enteroviruses	Viral risks associated with wastewater reuse: modelling virus persistence on wastewater irrigated salad crops	(Pettersson and Ashbolt, 2001)
Salad crops	Viruses	Microbial Risks from Wastewater Irrigation of Salad Crops: A screening - Level Risk Assessment Application of Microbial Risk Assessment to the Development of Standards for Enteric Pathogens in Water Used To Irrigate Fresh Produce	(Pettersson et al., 2001) (Stine et al., 2005a)
Raw Vegetables	Viruses	Quantitative Microbial Risk Assessment Models for Consumption of Raw Vegetables Irrigated with Reclaimed Water	(Hamilton et al., 2006)
Retail food	Noroviruses	Quantitative exposure model for the transmission of norovirus in retail food preparation.	(Mokhtari and Jaykus, 2009)
Coquina clams	HAV	Risk assessment in shellfish-borne outbreaks of hepatitis A.	(Pinto et al., 2009)
Irrigation water	virus	Potential chemical and microbiological risks on human health from urban wastewater reuse in agriculture. Case study of wastewater effluents in Spain.	(Munoz et al., 2010)

5.2. Critical review of data available (presented in this opinion) to conduct RA for FBV

Here, the availability of data which could potentially be used for risk assessment is discussed per step in the RA. With respect to the hazard identification, viral hazards and foods of concern have been identified.

For this opinion the FBV that have been identified as being of highest priority are NoV, HAV and HEV. Other hazardous FBV may be SARS coronavirus, avian influenza viruses and TBEV. Most emerging viruses can not easily be excluded from being foodborne (Duizer and Koopmans, 2008).

The implicated food items included shellfish (products), fresh produce, meat (products), and food handling environments (Table 8). With respect to fresh produce, the FAO/WHO expert meeting on the microbiological hazards in fresh fruits and vegetables recommended that leafy green vegetables should be considered the highest priority in terms of fresh produce safety from a global perspective²⁰.

Table 8: FBV and examples of implicated food items.

FBV	Examples of Implicated food item
NoV	Shellfish, raspberries, drinking water
HAV	bivalve molluscs, particularly oysters, clams and mussels, salad crops, as lettuce, green onions and other greens, and soft fruits, such as raspberries and strawberries
HEV	pork pies, liver pate, wild boar, under-cooked or raw pork, home-made sausages, meat (in general), unpasteurized milk, shellfish and ethnic foods
SARS coronavirus	food of animal origin
Tick-borne encephalitis virus	(raw) milk, yoghurt, butter and cheese
Nipah virus	Fruits
Avian influenza viruses	Duck blood

Food consumption statistics are available for consumption of berries and small fruits, water molluscs and crustaceans from the “Comprehensive European Food Consumption Database” (Appendix A). From these statistics, both data for individual member states and for Europe can be extracted.

²⁰ Viruses in Food: Scientific Advice to Support Risk Management Activities. Meeting Report Microbiological Risk Assessment Series, No. 13, 2009; <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=15&codcch=751>

Data on FBV in or on reservoirs/sources that may be used for or in contact with food during food production are abundant. For shellfish, numerous data on FBV in harvesting waters is known. For fresh produce, some data are available on FBV in irrigation water and manure but rather viruses were detected (presence/absence) than enumerated. For the food handling environment, FBV numbers in human and animal faeces are known. Less is known about the numbers of FBV, and their infectivity status, that are transferred from and between reservoirs/sources and foods. Some data are available for transfer between surfaces and hands, but virus transfer between food products and hands is largely unquantified. It is important to perform virus transfer experiments under controlled settings following specified protocols.

For high risk foods such as soft fruit and salad vegetables, irrigation water may be one of the sources of contamination and therefore this is one process that needs to be modelled. However, to estimate the concentration of virus on fresh products, it will be important to assess the volume of retained water on such products as a function of the duration of irrigation. Furthermore, the clinging of viruses to food products and wash-off during prolonged irrigation needs to be determined. However, for instance currently available data for lettuce is insufficiently accurate for use or not available.

On surfaces, hands and other environments, and once transferred onto food products, FBV may persist for prolonged periods of time (Table 9) enabling the viruses to reach the host. As for virus transfer experiments, virus occurrence data should be collected with inclusion of appropriate controls.

Table 9: Natural persistence of FBV

	NoV	HAV
Persistence on hands		Up to several hours
Persistence on surfaces	Up to 4-8 weeks	Up to several months
Persistence on food	Up to several days to months on fresh produce; up to several weeks in shellfish	Up to several days on fresh produce; up to several weeks in shellfish
Persistence in frozen foods		Up to several months

FBV may be reduced to a more or lesser extent by a diverse range of food treatment processes on which many reports are published (section 4.2). Efficiency of treatment processes for virus reduction was mostly assessed by use of indicator viruses such as MNV (murine NoV), FCV, bacteriophages. For QMRA, it is also needed to know which decontamination practices are used in food production, keeping in mind that lab experiments may give a different outcome as compared with virus reduction due to current practices in the field.

The presence of FBV on or in foods is proven with numerous reports on HEV in meat and HAV and NoV in shellfish and on fresh produce with clear adverse health outcomes especially in outbreak situations but not for individual cases or diffuse outbreaks. Moreover, the numbers of infectious, human pathogenic viruses present on or in foods is less well established.

The infectivity of FBV may be very high, e.g. exposure to one PCR detectable NoV unit (PDU) produces a probability of infection of 0.5 (Lindesmith et al., 2003; Teunis et al., 2008), up to a dose of 10^4 - 10^5 PDU for HEV in monkeys. Infectivity may be established by PCR for which the relation with infectious virus is generally unknown since for prominent FBV such as NoV and HAV no sensitive cell lines are known. In addition, dose-response relations are often established based on FBV in spiked water whereas no dose-relation for FBV on food is determined.

For QMRA, it is important to determine the risk outcome which could vary from infection risk, disease risk to disability-adjusted life years (DALY) as the health based target. Often immunity is not included which may be an omission.

Exposure to FBV may result in a higher incidence and a more severe disease outcome for vulnerable subpopulations such as the immunodeficient transplant recipients, those living with HIV/AIDS,

children, the elderly and pregnant women. NoV infection is common in all age groups but the incidence is highest in young children (<5 yrs). For HAV infection, it is clear that in high endemic areas due to induced life-long immunity upon exposure as a child, severe infections among adults are rare whereas in low endemic areas the disease occurs mostly in adulthood with the likelihood of developing severe symptomatic illness. Pregnant women are of high risk for developing severe courses of hepatitis E, which is reflected by the high mortality rate up to 25%. People with underlying disease, e.g. chronic liver disease, liver cirrhosis or a history of high alcohol consumption, are of higher risk of developing hepatitis E. Immunosuppressed transplant patients are of risk for developing chronic hepatitis E.

5.3. Conclusions on Risk Characterisation

There is a large variation in consumer habits across different member states, both in the percentage of the consumer sample that declared consumption of the specific food item, and in the average daily amount of product consumed. This would make the task of a EU risk assessment very complex.

Data on occurrence of FBV in reservoirs/sources like sewage, irrigation water, exist. However, sufficient quantitative data with respect to sampling size and numbers of viral particles are largely missing. In addition, there are no methods to determine infectivity (other than volunteer studies), and access to all the specific detail in studies is problematic. There is a requirement for specific targeted studies which follow guidance on data generation useful for QMRA.

Virus transfer between humans / animals / environment to foods and between foods are largely based on assumptions not experimental or field data.

Quantification of human pathogenic, infectious FBV on or in foods is largely lacking. Interpretation of PCR data is still under discussion.

Current human dose-response relations are largely insufficient for QMRA studies. For NoV the dose/response relations are based on RT-PCR data; for HAV and HEV, this is unknown, and only data based on monkey models are available.

Vulnerable subpopulations may experience higher disease incidence and more severe disease from exposure to FBV as compared with the general population.

5.4. Recommendations on Risk Characterisation

In order to quantify the efficacy of specific control options, it is necessary to build a risk assessment framework. This should be done for specific priority virus-commodity combinations, including consideration of the target population. A risk assessment will also help to identify data gaps, and to target research efforts.

However, also in the absence of a specific quantitative risk assessment, it is evident that certain control options could be implemented to reduce the risk.

For QMRA, viruses in foods need to be quantified. It is recommended to use RT-PCR for quantifying viral particles. Interpretation of the RT-PCR results with regard to risk of human infection and disease needs to be considered.

In the lack of volunteer studies, dose-response relations should be assessed from outbreak studies. In this situation, as much information as possible, related to for example, numbers, types, and infectivity of the virus in the suspected food commodity should be gathered. A guidance document for outbreak investigation for foodborne virus-related outbreaks could be drawn up to generate the type of data needed for QMRA.

There is a general lack in knowledge on how much disease is caused by the viruses discussed in this report, and on how much of this disease can be attributed to foodborne spread in comparison with

other possible transmission routes. Routine harmonised surveillance of viral outbreaks, and of virus occurrence in food commodities would be recommended to aid source attribution studies

6. Control options

Food may be contaminated with viruses at different steps in the food chain from primary production, to food processing, over retail (point of sale) to point of consumption, depending on the food commodity and methods of production.

Thus control methods for viruses in food will also differ between commodities depending on the risk of contamination of the specific products.

Apart from the general hygiene legislation and some (also more general) control measures for certain products like bivalve shellfish, no specific EC legislation including microbiological criteria exists for viruses in food. In the following section the following areas are covered:

- Summary of existing preventive measures in place according to current legislation
- Efficacy of current preventive measures
- Recommendations to improve efficiency of the control options in existing legislation
- Suggestions for additional/novel control options

6.1. Bivalve molluscs

6.1.1. Summary of existing preventive measures in place according to current EU legislation

Contamination of filter-feeding bivalve shellfish with human pathogenic viruses occurs through human faecal pollution of their growing areas. Sources can be diverse but frequently include direct continuous pipeline discharges of municipal sewage which may be treated to varying extents, periodic (intermittent) discharges from combined rainfall/sewage systems and emergency sewage overflows, leaks from ageing or poorly maintained sewage infrastructure, smaller discharges from individual properties e.g. septic tanks, discharges from boats, water courses (rivers, streams etc) contaminated higher in their catchments, etc. Risk management legislation for sanitary production of bivalve shellfish world-wide depends on assessment of the impact of such faecal pollution and then the prescription of food processing measures, if necessary, prior to placing the bivalves on the market. Legislative standards controlling permitted levels of faecal pollution world-wide utilise faecal indicator bacteria, for bivalve shellfish most countries employ either faecal coliforms or *E. coli*. These may be measured in the water column (USA system) or directly in the flesh of the bivalves (EU system). It is also possible to stipulate, on a precautionary principle, sea areas that should not be permitted for production based on the presence of known polluting sources such as sewage pipe discharges. However, this is not currently a feature of EU legislation which relies entirely on faecal indicator measurement to determine the applicable risk management controls.

The faecal indicator legislative standards governing production in the EU (the classification of production areas), and in third countries importing into the EU, are summarised in Table 10. Competent Authorities in EU Member States are required to define the location and boundaries of production (and relaying) areas and to classify the areas according to one of the three categories set out in Table 10. They are further required to establish a sampling (monitoring) programme, which should be representative, to ensure that bivalve molluscs harvested from the area comply with the established classification. If bivalves do not comply with the criteria the Competent Authority must close or reclassify the area. An essential first step prior to setting up a sampling programme is to survey the faecal pollution inputs, and their potential circulation within the production area, so that sampling points can be determined as representative according to scientific principles. This 'sanitary survey' is a requirement of both US and EU regulations. However, in the EU this only applies to areas

classified after 2006 and hence monitoring programmes for the majority of production areas in the EU (which were established prior to 2006) are not based on sanitary surveys. EU legislation does not contain detailed rules for implementation of monitoring programmes – for example key aspects, such as the required monitoring frequency, is not specified. An EU working group²¹ has drawn up detailed best practice guidance; however compliance with these rules is not mandatory.

Table 10: Summary of EU sanitation requirements for live bivalve mollusc production areas¹

Risk management measure required	EU Classification	Microbiological standard per 100g shellfish flesh and intravalvular liquid
Non required	Class A	all samples < 230 <i>E. coli</i> ²
Depuration or relaying ¹ or heat treatment by an approved method ³	Class B	90% ⁴ of samples < 4600 <i>E. coli</i>
Relaying over a long period ¹ or heat treatment by an approved method ³	Class C	all samples < 46,000 <i>E. coli</i>

¹ Regulation 854/2004. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:226:0083:0127:EN:PDF>

² Regulation 2073/2005 <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32005R2073:en:NOT>

³ Regulation 853/2004. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:139:0055:0205:EN:PDF>

⁴ transitional arrangement under EC 1666/2006.

<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:320:0047:0049:EN:PDF>

For the highest quality (class A) areas EU legislation does not require any further food processing to reduce the risk from faecal contamination. However, even such high quality areas are still occasionally associated with virus outbreaks (Dore et al., 2010; Maalouf et al., 2010a). For other more contaminated areas the food processing measures required by legislation are either depuration (self-purification) in tanks of clean seawater, relaying (self-purification in the natural environment) or commercial heat treatment (cooking) by an approved method. Bivalve molluscs that do not conform with any of the classification categories (i.e. that exceed class C levels) cannot be classified and hence cannot be placed on the market for human consumption. Some EU Member States have introduced a ‘prohibited’ classification to describe such areas. The operation of depuration, relaying and approved heat treatment processes by food business operators is subject to further detailed legislative rules (Reg. 853/2004). In all cases following such treatments the end-product prior to marketing must comply with a standard of <230 *E. coli* per 100g of shellfish flesh and intravalvular liquid (Reg. 2073/2005). The *E. coli* methods that may be used for both monitoring of production areas, and determining compliance with the end-product standard, are controlled by EU legislation (Regs. 854/2004, 2073/2005). Essentially a reference method is stipulated and either this must be used or an alternative method demonstrated to give equivalent results through a validation programme conducted according to international rules.

The EU-RL conducted a survey²² among EU Member States to establish the current status of classified production areas within the EU covering the numbers of areas classified and the percentage falling into the different classification categories. The survey showed that most EU Member States (but not all) have a full range of classifications with, overall, class B classifications predominating. Overall for the 3068 classified beds reported 40% were of class A status, 50% were class B status, 5% were class C status and 4% were assigned a prohibited status. This survey shows that the majority of European bivalve mollusc production areas do not fall into the cleanest (class A) category.

²¹ Microbiological Monitoring of Bivalve Mollusc Harvesting Areas - Guide to Good Practice: Technical Application. www.crlcefas.org/InformationCentre/docs/GPG_Issue3_Feb2007.pdf.

²² Comparison of bivalve mollusc harvesting area classifications under EC Regulation 854/2004 across EU Member States (2009). Dated 11/4/2011. www.crlcefas.org.

6.1.2. Evaluation of the efficacy of current preventive measures in the EU

The risk management measures prescribed by EU legislation vary in their effectiveness for reducing virus risk. Heat processing can be very effective if performed correctly (Reg. 853/2004) and in the UK following the introduction of revised criteria (raising core mollusc temperatures to 90°C for 90 seconds) hepatitis outbreaks from cockles harvested in the Thames estuary were brought under control (Lees, 2000). However, for products marketed live, depuration and relaying whilst effective at controlling bacterial infections (such as salmonellosis and typhoid), have been less effective for viruses. Depuration is a widely used commercial processing option in the EU. However, both epidemiological and laboratory studies show that depuration times and conditions currently used are inadequate to remove viruses (Lees, 2000; Richards et al., 2010). A key issue is that the legislative measure of acceptable quality of products placed on the market following depuration is compliance with an *E. coli* standard of <230 *E. coli* per 100g of shellfish flesh. Thus EU legislation does not specify key process criteria, such as duration of depuration or tank temperature, which may be critical for effective virus removal. Although, in line with general food law, depuration is required to be operated according to HACCP principles, the historic inability to measure virus contamination has left operators and authorities with little information on which to base virus removal criteria. In practise compliance with the *E. coli* requirement has been, and continues to be, the main determining factor and this is reinforced by the legislative text (Reg. 853/2004). Unfortunately there are many examples where bivalve shellfish causing outbreaks have been found to be fully compliant with the prescribed *E. coli* standard (Crocì et al., 2000; Le Guyader et al., 2006a; Le Guyader et al., 2010; Le Guyader et al., 2008; Lees, 2000; Sanchez et al., 2002). Alternate indicators such as coliphages, or adenovirus have been suggested (Dore et al., 2000; Formiga-Cruz et al., 2003), but none have yet been accepted. The main factors were insufficient scientific data underpinning the necessary correlation between presence of bacteriophages and viruses, together with the consequences for the industry.

Quantitative methods for NoV and HAV have only recently advanced to the stage where such studies can be performed. However, a recent field study following an outbreak (Westrell et al., 2010) used quantitative PCR to monitor NoV levels in oysters and suggested that virus contamination can be reduced to safe levels through a combination of extended relaying (at least 17 days) and depuration for an extended period (4 to 8 days) at elevated temperatures (15-17°C) (Dore et al., 2010). In this case NoV monitoring by PCR provided an effective assessment of virus risk and permitted effective risk management controls to be implemented. However some specific binding may influence these results depending on the strains and the shellfish species (Maalouf et al., 2010b; Zakhour et al., 2010). The use of PCR for monitoring virus risk is now the main focus for development of more effective control measures internationally.

6.1.3. Recommendations to improve efficiency of the control options in existing EU legislation

Bivalve molluscs present different risks for NoV depending on what is consumed. Those species consumed whole and raw clearly present a higher risk than those consumed cooked and/or eviscerated. However, EU legislation does not distinguish between these different levels of risk. In considering the recommendations below it may also be appropriate to consider whether they should be targeted only at higher risk products to ensure any additional regulatory burdens are kept proportional.

6.1.3.1. Classification monitoring and compliance

Currently EU Food Regulations (854/2004) require bivalve molluscs to be monitored and classified according to their *E. coli* content. However, the Regulations do not contain detail on how this should be performed. For example the Regulations do not stipulate a monitoring frequency, or how to select a monitoring point or how to interpret a data set in order to establish a classification. Consequently actual practices vary widely among EU Member States and will also vary in countries importing bivalves into the EU. In some cases this is likely to lead to differential health outcomes. An EU working group has elaborated agreed guidance on best practice for monitoring and classification. The general health status of EU bivalves could be improved by ensuring that such best practice was adopted more widely in the EU and third countries – particularly for high risk products. Sanitary

surveys are an assessment of pollution sources impacting a production area. They are an essential first step in a systematic approach for the design of monitoring programmes since they enable monitoring points to be located according to the identified pollution sources. However, this requirement was introduced into EU regulations in 2006 and areas classified before this time (the majority of production areas in the EU) do not require sanitary surveys unless they are re-classified. This can mean that the monitoring programmes for a large number of production areas are not established on a scientific basis. The health status of many production areas, particular where high risk species are produced, could be improved by making sanitary surveys compulsory for all areas. Currently there is no requirement in EU legislation to report on the hygiene status of production areas. Public domain publication (e.g. an annual reporting requirement) of classifications, sanitary survey assessments, and associated monitoring data, would lead to improved transparency in sanitary assessments and would thus probably improve the health quality of intercommunity trade and also of imports into the EU, which have been responsible for a number of outbreaks. The *E. coli* standards stipulated in EU legislation regarding classification criteria are essentially arbitrary risk management levels. The sanitary quality of bivalves could be improved by lowering the pollution (*E. coli*) levels permitted. This is probably most relevant for depurated high risk products (e.g. oysters) originating from class B production areas since depuration is known to be problematical in removing enteric viruses. The upper level of class B is relatively high, and exceeding this level by 10% is also permitted. Another way of improving the situation would be to specify in class A and B (low level of contamination) the critical points [i.e. old sewage infrastructures, ageing municipal treatment plant or other (important watershed sloop, presence of agriculture spreading)] leading to possible intermittent introduction for a short time of faecal discharges.

6.1.3.2. Depuration and relaying

Under EU legislation the only permitted treatments for class B or C bivalves molluscs placed live on the market are relaying and/or depuration. Both essentially rely on continuation of the normal mollusc filter-feeding processes using clean seawater to flush or purge out faecal contaminants. Relaying is conducted in the natural environment, depuration (also termed purification) in tanks. Molluscs need to be in good condition and with the correct physiological conditions to purify successfully. Hence, it is important to ensure that critical parameters such as temperature, salinity, oxygen levels, etc are well controlled. This creates a significant problem for regulation since it is not possible to stipulate in legislation the great variety of critical physiological parameters for the range of European species and habitats. Instead, EU legislation relies heavily on the use of compliance with the *E. coli* end-product standard (<230 *E. coli* per 100g) for determining acceptable practices. The key problem here is that viruses are removed much more slowly than bacteria during depuration and relaying and hence molluscs compliant with the *E. coli* standard may still contain enteric viruses and cause outbreaks. This was noted in a previous opinion by the SCVPH which issued an opinion on Norwalk-like viruses (NLVs, noroviruses) on 30-31 January 2002. In that opinion it concluded that the conventional faecal indicators are unreliable for demonstrating the presence or absence of NLVs and that the reliance on faecal bacterial indicator removal for determining shellfish purification times is unsafe practice. Unfortunately this is still the case. Depuration remains a widely utilized food process within the EU for treatment of class B bivalve molluscs prior to placing on the market. Many outbreaks of virus illness, including recent ones, can be traced to depurated shellfish (see 6.1.2) hence this process as currently performed under EU legislation is demonstrably not providing adequate levels of public health protection. However, there are indications that relaying, since it can be performed for much longer periods, is capable of reducing virus levels and can be an effective process when used in combination with depuration and when the process is monitored using PCR (Dore et al., 2010). Since methods for detecting NoV and HAV are now available a much more effective approach would be to require food business operators to determine the depuration and relaying operating procedures incorporated into their HACCP plans according to removal of human enteric viruses rather than *E. coli*.

6.1.3.3. EU environmental legislation.

It is fundamentally important to protect and improve the quality of seawater in production areas since food processing methods for removing contamination from live molluscs are largely ineffective. The importance of this is emphasised by a recent survey conducted by the EU-RL among EU Member States showed that the majority of European bivalve mollusc production areas do not fall into the cleanest (class A) category. Environmental legislation is necessary to control pollution sources as diverse as municipal sewage discharges, combined storm overflows, discharges from boats and individual dwellings, etc. Currently bivalve mollusc production areas received protection through the Shellfish Waters Directive²³ (2006/113/EC) which sets a guideline microbial standard. However, this Directive is due to be repealed in 2012 and its measures subsumed into the Waters Framework Directive²⁴ (2000/60/EC). Unfortunately this directive does not contain any specific microbiological standards for shellfish waters and therefore it is difficult to see how the current safeguards can be maintained. A mandatory *E. coli* and/or virus standard in the Water Framework Directive for designated bivalve shellfish areas (particularly for high risk species), coupled with a specified time period for improvement of areas failing the standard, would considerably improve the overall health status of EU bivalve molluscs.

6.1.4. Suggestions for additional/novel control options currently not covered in EU legislation

6.1.4.1. Risk based.

Since it is clear that not all bivalve molluscs pose equal risks it is suggested that, if additional measures are considered, these should be targeted at 'high risk' products to avoid inappropriate regulatory burdens. The bivalve species consumed whole and raw (or very lightly cooked) very clearly present a much higher risk than those consumed well cooked and/or eviscerated. Such high risk products are associated with the overwhelming majority of the reported enteric virus related outbreaks. However, a difficulty is how to identify such products in order to target additional risk based food control measures. Possible options might be:

- (i) Based on traditional consumption patterns for each species and what is marketed i.e. products sold whole and traditionally eaten raw (e.g. oysters).
- (ii) Based on a determination by the Competent Authority in each Member State. For example the CA could list production areas and species classed as 'high risk' according to epidemiological data or other risk information they might consider relevant (guidance criteria could be established).
- (iii) Based on consumption advice to consumers. For example products labelled as 'cook well before consumption' (with associated cooking advice) could be considered as lower risk than those not so labelled.

6.1.4.2. Criteria for viruses.

Since robust methods are now available for direct detection of NoV and HAV in molluscs it is now feasible to introduce hazard-based controls for viruses. It is suggested that these should be targeted at high risk products as defined above. The criteria could be introduced for the marketed product and/or for monitoring of production areas. Taking into consideration monitoring data (quantification of virus), the introduction of criteria for viruses into EU legislation (for high risk products) would have an effect on avoiding that the most heavily contaminated products are placed on the market, therefore reducing the human exposure and public health risks posed by bivalve molluscs (see section 7.2). A risk management decision would be to decide on the threshold level that should be adopted.

²³ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:376:0014:0020:EN:PDF>

²⁴ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:327:0001:0072:EN:PDF>

6.1.4.3. Reduction of contamination levels associated with products from classified production areas.

Since many outbreaks are associated with high risk products harvested from classified production areas specific targeting of additional measures in this area could help reduce virus risk. Currently, for example, there are no legislative controls preventing harvesting in the immediate vicinity of sewage discharges. Such practices present obvious high risks and are clearly unsatisfactory. For example extended legislation could require a mandatory harvesting prohibition zone round all human discharge sources (a minimum distance or dilution criteria could be established). Such measures are already incorporated into bivalve mollusc sanitation legislation in countries outside of the EU. However, this could only be applied if a sanitary survey had been performed in the production area and thus the pollution sources were documented. This measure would thus also require sanitary surveys to be performed for all production areas (see above) as a first step. Sanitary surveys also provide the fundamental pollution impact data needed to consider proactive management of bivalve production areas. In some countries, for example the USA, production areas are actively managed to avoid harvesting during pollution episodes such as during periods of heavy rainfall. Thus contaminated products can avoid being placed on the market. European research projects (Seafoodplus, Food-CT-2004-506359) and Virus Safe Seafood, QLK1-1999-00634) have demonstrated the validity of this approach in a European context which has significant potential benefits for both producers and public health.

6.2. Fresh produce

6.2.1. Summary of existing preventive measures in place according to current EU legislation

Fresh produce *in primary production* needs to fulfil the general rules of hygiene laid down in Regulation EC No 852/2004²⁵

- Food business operators producing or harvesting plant products are to take adequate measures, as appropriate:
 - to keep clean and, where necessary after cleaning, to disinfect, in an appropriate manner, facilities, equipment, containers, crates, vehicles and vessels;
 - to ensure, where necessary, hygienic production, transport and storage conditions for, and the cleanliness of, plant products;
 - to use potable water, or clean water, whenever necessary to prevent contamination;
 - to ensure that staff handling foodstuffs are in good health and undergo training on health risks;
 - as far as possible to prevent animals and pests from causing contamination;
 - to store and handle wastes and hazardous substances so as to prevent contamination;
 - to take account of the results of any relevant analyses carried out on samples taken from plants or other samples that have importance to human health;
- Good Agricultural Practices (GAP)
- Use of potable water or clean water when necessary to prevent contamination (no criteria)
- Can use guidelines set in Global GAP (is not legally obliged)

²⁵ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32004R0852:en:NOT>

Fresh produce **processing** needs to fulfil Reg. 852/2004:

- Good Manufacturing Practices (GMP)
- Use of potable water or clean water when necessary to prevent contamination (no criteria)
- HACCP plan

Microbiological Criteria are set by EU 2073/2005 and those covering fresh produce (processed) are:

- Food safety criteria for sprouted seeds, pre-cut fruits and vegetables, and unpasteurised fruit and vegetable juices. *Salmonella*: absence/25g.
- Process hygiene criteria for pre-cut fruits and vegetables, and unpasteurised fruit and vegetable juices *E. coli*: m= 100/g and M=1000/g

The conclusion is that no specific EC legislation including microbiological criteria exists for viruses in fresh produce and the requirements to food business operators producing or harvesting plant products are very general in nature and leave room for subjective interpretation i.e. use potable or clean water whenever necessary.

Some International guidelines exist (not EU legislation), as an example Codex Alimentarius has a code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003)²⁶. The code addresses Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) that will help control microbial hazards associated with all stages of the production of fresh fruits and vegetables from primary production to packing. The code provides a general framework of recommendations to allow uniform adoption by this sector rather than providing detailed recommendations for specific agricultural practices, operations or commodities.

The fresh fruit and vegetable industry is very complex. Fresh fruits and vegetables are produced and packed under diverse sanitary conditions. It is recognized that some of the provisions in the code may be difficult to implement in areas where primary production is conducted in small holdings, in both developed and developing countries and also in areas where traditional farming is practiced. Therefore, the code is, of necessity, a flexible one to allow for different systems of control and prevention of contamination for different groups of commodities. Since the code is directed towards controlling microbiological hazards in general it is also applicable for controlling viruses of both human and animal origin.

In 2007, a FAO and WHO Expert Meeting on the microbiological hazards associated with fresh produce took place²⁷. The expert meeting ranked leafy green vegetables as the group of most concern in relation to amongst other frequency and severity of disease and size and scope of production. The second priority of concern was the group of berries, green onions, melons and tomatoes. All these types of fruits and vegetables have been implicated in foodborne viral infection. A proposed draft annex on fresh leafy vegetables (Annex to the Code of Hygienic Practice for Fresh Fruits and Vegetables) is now being elaborated.

Controlling viruses in fresh produce clearly needs a food-chain approach, taking into account all aspects from primary production to consumption. This includes consideration of the inputs to primary production, which include the farm environment (soil, wildlife etc), irrigation water source, manure etc. In addition, the workers (growers, pickers) and transport (open transportation may provide

²⁶ www.codexalimentarius.net/web/standard_list.do?lang=en

²⁷ Viruses in Food: Scientific Advice to Support Risk Management Activities. Meeting Report Microbiological Risk Assessment Series, No. 13, 2009; <http://apps.who.int/bookorders/anglais/detart1.jsp?sesslan=1&codlan=1&codcol=15&codcch=751>

contamination opportunities) from the field to the packing and processing houses are a consideration at this stage. All represent potential sources of contamination and their relevance to the particular commodity of concern may need to be assessed. Also at later stages during i.e. packing the possibilities for contamination from handling needs to be taken into account.

In the Code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003) there is a focus on water quality to be used in primary production as irrigation etc, for the use of manure, biosolids and other natural fertilizers and for personnel health, hygiene and sanitary facilities. However as earlier mentioned the code provides a general framework of recommendations to allow uniform adoption by this sector rather than providing detailed recommendations for specific agricultural practices, operations or commodities.

Proposed draft guidelines on the application of general principles of food hygiene to the control of viruses in food is being elaborated in Codex²⁸ Committee on Food Hygiene and is at step 3. The primary purpose of these guidelines is to minimize the risk of illness arising from the presence of human enteric viruses in foods, and more specifically from NoV and HAV in foods. These guidelines will have a specific focus on controlling viruses in shellfish and fresh produce and include specific guidance on food handling.

There is no EU legislation in place regarding irrigation water.

6.2.2. Evaluation of the efficacy of current preventive measures in the EU

No specific EC legislation including microbiological criteria exists for viruses in fresh produce. The requirements to food business operators producing or harvesting plant products are very general in nature and leave room for subjective interpretation i.e. use potable or clean water whenever necessary.

Also there are no specific standards regarding the quality of irrigation water. General principles in annex C of directive 2008/98/EC²⁹ are not very useful, and more specific standards for irrigation water would be needed.

It is expected that the existing *E. coli* process hygiene criteria for certain fresh produce products (seeded sprouts and pre-cut fruit and vegetables) may contribute towards the sanitation level of these products. However, it is questionable whether this has sufficient impact on the risk of viral contamination.

6.2.3. Suggestions for additional/novel control options currently not covered in EU legislation

6.2.3.1. Consideration of microbiological criteria

Methods exist for detecting viruses in fresh produce, and studies on the occurrence of viruses in fresh produce are ongoing. Viruses can be detected in fresh produce, but prevalence studies are limited, and quantitative data on viral load is scarce making establishment of microbiological criteria for these food categories difficult.

6.2.3.2. Irrigation water quality

Solely standard setting as control option for virus safe use of irrigation water is not sufficient due to problems associated with feasibility, meaning of indicator detection for viruses and cost of monitoring. The WHO Guidelines for the safe use of wastewater, excreta and grey water in agriculture and aquaculture³⁰ are based on a sustainable and promising risk analysis approach, which is recognized internationally as the fundamental methodology underlying the development of food safety standards that both provide adequate health protection and facilitate trade in food. Adherence to the WHO

²⁸ www.codexalimentarius.net/download/report/753/REP11_FHREVe.pdf

²⁹ <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:312:0003:0030:en:PDF>

³⁰ WHO Guidelines for the safe use of wastewater, excreta and greywater. ISBN 92 4 154686 7.

Guidelines in the application of wastewater, excreta and grey water for the production of food products destined for export will help to ensure an unencumbered international trade of safe food products. Clearly, this requires a sound monitoring process to ensure compliance with the risk management measures and appropriate quality control along the way from wastewater generation to produce consumption. The procedures for this monitoring process should be embedded into national policies and regulations for water quality that also apply to drinking-water quality, safe recreational waters (bathing water profiles), safe shellfish growing areas (shellfish sanitary surveys) and the concept of water safety plans in general³¹.

Guidelines/legislation related to the use of faecal contaminated water and/or manure on fresh produce in a specified period before harvest is necessary in order to avoid contamination of fresh produce with viruses through this source.

6.2.3.3. Sewage treatment

Wastewater may affect food production chains in several ways through discharges of treated and untreated wastewater onto surface waters used for irrigation purposes or at risk of flooding the production site. Primary and secondary wastewater treatment processes typically reduce virus loads by 1-2 log₁₀-units (Lodder and de Roda Husman, 2005; van den Berg et al., 2005). Efficiency of wastewater treatment processes may be upgraded by use of novel processes such as membrane bioreactor (MBR). Viruses in general show a high removal rate using MBR and can achieve better microbial removal in far fewer steps than conventional activated sludge process with advanced tertiary treatment (Zhang and Farahbakhsh, 2007). A recent paper on adenovirus removal by full-scale MBR showed an average HAdV removal of 5.0 +/- 0.6 log₁₀-units, however, leaving about 10³ viral particles/L in the MBR effluent (Kuo et al., 2010). For indigenous somatic coliphages, the MBR systems achieved 2.6 to >3.4 log₁₀-units in another study (Hirani et al., 2010).

6.2.3.4. Decontamination of fresh produce

Possible treatments and their effect to decontaminate fresh produce are described in section 4.2. It should be noted that the effect of a treatment varied from one study to another. This can be explained by the different experimental set-up, type of produce and the tested virus strains.

In general, washing of produce resulted in a maximum 1 to 2 log reduction, similar as for bacterial pathogens. Currently, chlorine is the most widely used sanitizer in the food industry. The use of chlorine (NaOCl) at a concentration of 200 mg/L to treat produce enabled an additional 1 log reduction compared to washing produce in plain water.

A possible alternative is peracetic acid (PAA). PAA is a mixture of acetic acid and H₂O₂ in an aqueous solution. It outranges the oxidation potential of chlorine. Moreover, it is shown to be hardly influenced by organic compounds present in lettuce wash waters. The use of 150-250 mg/L PAA was shown to be needed to induce at least an additional 1 log reduction compared to washing produce in water. With all of these decontamination procedures, effect on produce (nutritional and sensorial aspects) and consumer acceptance are important criteria for use.

Despite the efficacy of decontamination treatments (generally between 1 and 3 log reduction of the viral load), the probability of infection cannot be reduced to zero if an initial high contamination level is present on produce. Decontamination procedures can be useful to lower the viral intake and can even decrease the probability of infection to 0% for fresh produce having an initial low viral contamination level.

Regulation 853/2004 of the European Parliament and Council constitutes the legal basis for the use of substances other than potable water or clean water to remove surface contamination from foods of animal origin intended for human consumption. In general, water coming in contact with food should

³¹ WHO Guidelines for Drinking-Water Quality. ISBN 978 92 4 154761 1.

be of “drinking water quality”. There is not yet a clear defined European legislation about the use of processing aids in wash water in the fruit and vegetable industry. In some countries, processing aids are approved to keep the microbiological quality of wash water under control. In this case, a processing aid is permitted if it is demonstrated that no unacceptable residues remain on the end product.

Taking into consideration the legislative restrictions and the minimal effect of decontamination strategies (minimal processing), attention towards preventive measures (GAP, ...) would be preferred rather than relying upon the removal of viruses from fresh produce.

Though the efficacy of many alternative sanitizers such as chlorine dioxide (ClO₂), hydrogen peroxide (H₂O₂), PAA and ozone (O₃) have been studied with some of the human pathogenic viruses, their use is not often routinely practiced.

6.2.3.5. Control options related to food handler

Personal hygiene of food handlers is critical. Food handlers should be aware of the high infectivity and transmission routes of enteric viruses such as NoV and HAV. Food handlers with clinical symptoms of gastroenteritis (diarrhoea and/or vomiting) or with symptoms of acute hepatitis (fever, headache, fatigue combined with dark urine and light stools, or jaundice), should be excluded from food handling and should not be present in the primary production area, so as to reduce the likelihood of transmission of NoV and HAV. Worker(s) should leave the primary production area directly after vomiting or the first event of diarrhoea. Any person with symptoms of acute hepatitis should seek medical advice.

When one of the staff members arrives at work or calls in with symptoms of gastroenteritis or hepatitis, other staff members may also be (asymptomatically) infected and all staff members should comply with strict hand hygiene measures. Compliance with good hand hygiene remains important at all times. In the case that one or more staff members complains of or is diagnosed with acute hepatitis, the whole staff should seek medical advice.

In the case of gastroenteritis, staff should only be allowed to return to work after a period without symptoms of diarrhoea and vomiting (e.g., period of 48 hours) or in case of hepatitis, staff should only be allowed to return to work after disappearance of jaundice and after having a complete medical examination.

As shedding of viruses such as NoV or HAV may continue after their symptoms have subsided (post-symptomatically) (e.g., NoV can be present in the stool on average for 4 weeks up to 8 weeks), these persons should comply with strict hand hygiene instructions (i.e. thorough hand washing with soap and running water, and preferably drying hands with disposable towels), and they should preferably use a separate bathroom where this is possible.

Vaccination against hepatitis A could be recommended to immunize food handlers to reduce the risk of viral contamination of the food, taking into account the epidemiological situation and/or immune status of the local population, e.g. where HAV is endemic or the population has low immunity.

Training should be provided to food handlers and managers.

Staff working as harvesters in primary production should be provided with sanitary facilities and procedures put in place so that these are used correctly.

6.3. Products of animal origin (meat)

6.3.1. Summary of existing preventive measures in place according to current EU legislation and evaluation of the efficacy of current preventive measures in the EU

HEV can be transmitted through consumption of products of animal origin, especially through consumption of meat. This virus may circulate in the blood at the time of slaughter and may be present in liver or meat. However, no specific legislation for HEV is currently in place.

Regulation related to hygienic measures for foods of animal origin and control of products of animal origin for consumption is laid down in EU legislation 853/2004 and 854/2004. Mandatory ante-mortem inspections of individual animals and post-mortem inspection and incision of individual carcasses are sufficient for prevention of certain meatborne zoonoses such as bovine tuberculosis, brucellosis and certain parasitic infections. Ante-mortem and post-mortem inspection is however not efficient for detection of HEV which may be present in liver or meat at the time of slaughter without causing visible changes in living animals or in their organs.

In addition, traditional meat inspection is not efficient in the control of zoonotic agents (*Salmonella*, *Campylobacter*, HEV), which may be present in animal feces. In the EU legislation measures to avoid or to reduce faecal contamination of carcasses exist and also performance criteria for *Enterobacteriaceae* and *Salmonella* are in place. All measures taken to avoid or to reduce faecal contamination will also have an impact on possible surface contamination of carcasses with HEV derived from faeces. However, the relative importance of faecal surface contamination for transmission of HEV is not known so far.

6.3.2. Suggestions for additional/novel control options currently not covered in EU legislation

Presently the only efficient control option for HEV infection from consumption of meat or liver is sufficient heat treatment

General suggestions for heat treatment of risk products could be useful, however, the distinct time/temperature conditions for inactivation of HEV in meat and meat products are not known so far making general suggestions difficult (Emerson et al., 2005). Improved kitchen hygiene may prevent transfer of HEV from raw meat to products thereafter eaten raw; however, the relative importance of this transmission route is not clear so far (see below). Education campaigns for high risk groups should be initiated, especially for people with underlying liver disease or for immunosuppressed persons as scientific evidence implies that the clinical course of hepatitis E is more severe in these groups even in Europe. Long-term heating of meat and liver derived from wild boars and pigs might be suggested for these risk groups. Scientific evidence of severe clinical hepatitis E courses in pregnant women is currently only available for the endemic regions in the developing countries; however, the number of well documented clinical hepatitis E courses in pregnant women in Europe is very low thus the possibility of underestimation is present. In order to precautionary protect pregnant women, this group should be included as risk group for hepatitis E in the education campaigns.

The option to prevent HEV introduction into pig farms and thereby reduce the proportion of HEV infected pigs at the time of slaughter is hampered so far by the limited knowledge about the distinct transmission pathways of HEV in pigs (Bouwknegt et al., 2009; Kasorndorkbua et al., 2004).

Data needs:

Generally, the existing data gaps on HEV epidemiology first of all claim to enhanced research activities, mainly on the following fields of interest:

- Assessment of the importance of the distinct HEV transmission routes to humans and the identification of risk factors for human hepatitis E. This may be done by conduction of more well documented case/control studies. This should also include the assessment of the importance of surface contamination of meat by pig faeces and of cross-contamination in the kitchen for transmission of HEV.
- Assessment of distinct transmission pathways of HEV between pigs. This should also include considerations on opportunities to create HEV-free pig farms.
- Assessment of the distribution of infectious HEV in meat and meat products in Europe. To this end, standardized (quantitative) detection methods have to be developed.

- Assessment of the transmissibility of HEV via meat and meat products. This includes the determination of the oral infectivity of HEV, which should enable an estimation of the importance of distinct transmission pathways as well as define the requirements for measures leading to reduction of infectious HEV.

- Assessment of the resistance of HEV to food processing, including heat, pH and distinct processing techniques for meat products.

The results of the research activities should be a basis for a more profound risk assessment including science-based suggestions for novel control options.

6.4. Ready-to-eat foods

The most probable source of contamination of RTE foods is the food handler. Contamination of food by an infected food handler can be prevented by strict enforcement of hygiene measures. These activities are covered by regulation (EC) No 852/2004 on the hygiene of foodstuffs, which provides general rules for food business operators for the production and processing of all food throughout the food chain.

An infected food handler not respecting hygiene regulations can contaminate surfaces (e.g. tools used for preparing RTE foods such as knives, spoons, chopping boards, etc). As a consequence, transmission of NoV to RTE foods might be possible from contact with contaminated surfaces as well.

6.4.1. Summary of existing preventive measures in place according to current EU legislation

Regulation EC No 852/2004 regarding the hygiene of foodstuffs describes general hygiene requirements; some key-elements:

- General requirement for food premises: adequate number of flush lavatories and washbasins. Need for materials to clean hands and hygienic drying. Separation of facilities to wash food and hands.
- Food premises and materials (wall and floor surfaces, ceilings, food contact surfaces,...) should be designed allowing adequate maintenance, cleaning and disinfection
- Adequate facilities for cleaning and disinfection (storage and equipment)
- Equipment which come in contact with food should be effectively cleaned, where necessary disinfected.
- Personal hygiene:
 - o High degree of personal cleanliness, wearing suitable clothing;
 - o No person suffering from, or being a carrier of a disease likely to be transmitted through food (infected wounds, skin infections, sores, diarrhoea) is permitted to handle food. Immediately report illness, symptoms.
- Food business operators have to ensure by training programmes:
 - o that food handlers are supervised and instructed and/or trained in food hygiene matters commensurate with their work activity;
 - o that those responsible for the development and maintenance of the procedure based on HACCP principles and for the operation of relevant guides have received adequate training;
 - o compliance with any requirements of national law concerning training programmes for persons working in certain food sectors.

6.4.2. Evaluation of the efficacy of current preventive measures in the EU

General hygiene requirements are not specific for viruses.

6.4.3. Recommendations to improve efficiency of the control options in existing EU legislation

The effect of hand sanitizers and surface disinfectants should be evaluated towards the virucidal activity (non-enveloped viruses).

Hand sanitizing-agents have not been shown to be able to completely eliminate enteric virus infectivity from hands. Consequently, it is conceivable that considerable numbers of infectious viruses will remain when hand sanitizers are used instead of traditional hygienic hand washing with streaming water and soap, followed by drying using disposable towels.

Most surface *disinfectants* lack efficacy (i.e., consistently cause less than a 3 log reduction in infectivity) against enteric viruses at manufacturers' recommended concentrations and exposure times. In fact, it is well recognized that the majority of chemical disinfectants currently used in both institutional, domestic environments, and in the food industry do not effectively inactivate HAV. New compounds and/or methods can be considered if they show a virucidal activity of $>3 \log 10$ for non-enveloped viruses in standardized carrier tests.

6.4.4. Suggestions for additional/novel control options currently not covered in EU legislation

A working group of Codex Alimentarius³² is drafting a detailed guidance document specifying how the general principles laid out in the current legislation can be applied to control viral contamination of food:

Food handlers with clinical symptoms of gastroenteritis (diarrhoea and/or vomiting) or with symptoms of acute hepatitis (fever, headache, fatigue combined with dark urine and light stools, or jaundice), should be excluded from food handling and should also be excluded from being present in the food handling area to reduce the likelihood of transmission of enteric viruses, such as NoV and HAV, that may be the underlying cause of the symptoms of gastroenteritis or hepatitis, respectively. A person should leave the food handling area directly after vomiting or on the first event of diarrhoea. A person with symptoms of hepatitis should seek medical advice.

In the case of gastroenteritis, workers should be allowed to return to work only after a period without symptoms of diarrhoea and vomiting (e.g., a period of 48 hours) or in the case of hepatitis, after disappearance of jaundice and a medical examination and education on contagiousness.

As the shedding of NoV or HAV may continue post-symptomatically, these persons must be reminded of the need to comply with strict hand hygiene requirements (i.e. thorough hand washing with soap and running water) and they should preferably use a separate bathroom if available.

When one of the staff members calls in with symptoms of gastroenteritis or hepatitis, other staff members may also be (asymptomatically) infected, and subsequently the establishment should evaluate the potential for other staff members to be infected and all staff members should comply with strict hand hygiene. Compliance with good hand hygiene remains important at all times. Moreover, if one or more staff members complains of or is diagnosed with acute hepatitis, the whole staff should seek medical advice. Acknowledge the fact that when a family/house member of one of the staff members has symptoms of gastroenteritis or hepatitis, the staff member may also be (asymptomatically) infected, and/or serve as a fomite carrying infectious virus on their person. Such staff members should, therefore, also comply with strict hand hygiene, or in the case of hepatitis also seek medical advice.

³² www.codexalimentarius.net/download/report/753/REP11_FHREVe.pdf

Hands should be washed before handling of food. The most effective way of preventing spread of virus is thorough hand-washing. Hands should be lathered with soap then washed for 20 seconds with running water. Hands should be dried preferably with disposable (paper) towels for a further 20 seconds². Everyone should always wash his or her hands after using the toilet or after being in contact with faecal matter (also after changing diapers, cleaning toilets) or after being in contact with vomit. The use of disposable hand towels should be encouraged.

In addition, money, tickets etc., should not be handled at the same time as food when wearing gloves. When this is not possible, new gloves should be put on before preparing food.

Vaccination against hepatitis A could be used to immunize food handlers to reduce the risk of viral contamination of the food, taking into account the epidemiological situation and/or immune status of the local population, e.g. where HAV is endemic or the population has low immunity.

In addition, establishments should also have a procedure for the **disinfection of surfaces** possibly contaminated with enteric viruses, such as NoV or HAV. Disinfection, preceded by cleaning, should take place after each vomiting event in premises or rooms, after reported symptoms of gastroenteritis (diarrhoea and/or vomiting) or symptoms indicative of hepatitis (fever, headache, fatigue combined with dark urine and light stools, or jaundice) of one or more of the employees. Cleaning and disinfection should include all surfaces both in the bathroom and (as a preventive measure) in food production areas (e.g., equipment, utensils, telephones, keyboards, etc.), as viruses in vomit, aerosols and faecal matter are persistent and can stay infectious for a long period. For surface disinfection, solutions of ≥ 1000 ppm free chlorine consistently show over 3 log reduction in viral infectivity within 5 min at room temperature. Freshly constituted hypochlorite solutions (e.g. using tablets) are preferable. The solution is corrosive, and needs to be thoroughly removed afterwards. Adequate precautions should be taken during cleaning or disinfection of rooms, equipment or utensils, to prevent food being contaminated by wash water, detergents and disinfectants. Food preparation should only begin after thorough disinfection has taken place. UV irradiation at >40 mWs/cm² (=mJ/cm²) causes > 3 log 10 reduction of feline calicivirus (FCV) and murine norovirus (MNV), which have been used as models for human NoV and HAV, and this treatment can be considered for reducing viral infectivity on surfaces, in aerosols and in water.

Ideally, disposable gloves, a disposable facemask and a disposable apron should be worn during cleaning and disinfection by ideally a person trained in cleaning-up infectious material, because of the exposure to highly infectious pathogens. Any spillage or contamination with faeces or vomit should be dealt with immediately, and food handling in the same area(s) should be stopped. If the indicated area is very large this might not be realistic, and in this case food handling only in the surrounding area should be stopped. Dispose of any food possibly contaminated by vomit particles or by aerosols containing vomit particles. Any food handled by the ill person during that day (or the day before (NoV), or longer (HAV)) could be at risk, and disposal of implicated product should be considered. Absorbent material such as paper towels and tissues may be used to limit the spread of liquid soiling and subsequently disposed of. Surfaces should be cleaned to ensure effective disinfection.

Training programs should contain information on the following: the potential for contaminated food to be a vehicle for virus transmission; the potential sources and routes of transmission of human enteric viruses; the incubation periods of foodborne viruses, specifically NoV and HAV; the duration of virus shedding even after recovery from clinical symptoms; the possibility of asymptomatic shedding; the infectivity of vomit; cleaning and disinfection of contaminated surfaces; the need for strict compliance to hand washing instructions at all times, and the need for washing of hands after being in contact with faecal or vomit matter. Training must also emphasise that if a staff member calls in sick, it is likely that other members may be (asymptomatically) infected too, and, in addition, if a household member is ill, it is likely that the staff member may be (asymptomatically) infected too. Staff members should also be taught to stay away from work and not have direct contact with any ready-to-eat food if they have symptoms of NoV or HAV infection. Training must also emphasize the need to keep children away from food growing fields and food preparation areas in HAV endemic areas.

6.5. Vaccination (humans)

Inactivated HAV vaccines are available since the early 90s and provide long-lasting immunity against hepatitis A infection. The immunity is largely related to the induction of high titers of specific antibodies. Thanks to the existence of a single serotype of HAV, these vaccines are of high efficacy. These vaccines consist of viruses grown to high titers in cell culture, purified, inactivated with formalin and adsorbed to an aluminium hydroxide adjuvant, making their economic cost quite high. This is the reason why many discrepancies already exist on their universal use in massive vaccination campaigns. Countries with previous intermediate endemicity of HAV such as Israel or some Autonomous Communities of Spain such as Catalonia, or some States of United States have performed studies on the beneficial impact of child vaccination on the overall incidence of hepatitis A concluding that the immunization is medically (Dominguez et al., 2008; Wasley et al., 2005) and economically (Dagan et al., 2005) justified. In contrast other countries in a similar situation such as Italy do not recommend at present the implementation of such a measure in terms of cost-benefits (Franco and Vitiello, 2003). In this context is quite evident that high endemic countries that usually have low economic incomes do not regard the vaccination against hepatitis A as a primary policy (Teppakdee et al., 2002). Although several attenuated vaccine candidates have also been attempted, due to the successful use of inactivated vaccines, its development is hardly plausible.

As a general rule in low and intermediate endemic regions, where paradoxically the severity of the disease is high, vaccination against hepatitis A should be recommended in high-risk groups, including travellers to high endemic areas, men having sex with men, drug users and patients receiving blood products. In addition, the inclusion of hepatitis A vaccines in mass vaccination programs in those countries receiving high numbers of immigrants from endemic countries is particularly advisable.

Nevertheless, the quasispecies replication pattern of HAV (Sanchez et al., 2003a) could lead to the selection of new antigenic variants escaping immune protection, in populations with continued exposure to the virus (Aragones et al., 2008). Hence in these conditions, mass vaccination programs in highly endemic areas are controversial.

7. Microbiological criteria or Microbiological testing as a control option

7.1. Introduction to microbiological criteria.

Food safety needs to be ensured through the structured approach of HACCP which requires producers to identify hazards and eliminate or control them at Critical Control Points (CCP) together with controls at primary production, GHP and GMP and controlled conditions of distribution and sale. Microbiological testing and microbiological criteria is only one of several control options and should not be considered without other aspects of EU food legislation, in particular HACCP principles and official controls to audit food business operators' compliance.

A microbiological criterion consists of specific elements such as the analytical method, the sampling plan, microbiological limit(s), the specified point of the food chain where the limit(s) apply, the number of analytical units that should confirm to the limit(s) and the actions to be taken when the criterion is not met. Microbiological Criteria (MC) should be scientifically based

Microbiological criteria are useful for validation and verification of HACCP-based processes and procedures, and other hygiene control measures. In addition microbiological criteria are used to assess the acceptability of a batch of food, including the circumstances where there is insufficient knowledge of production conditions e.g. at port of entry. The microbiological criteria does not mean that all food batches have to be tested, but clarifies how the test results should be interpreted from a food batch, and the risk management consequences.

In EU legislation, they are also used as a way to communicate the level of hazard control that should be achieved. Meeting microbiological criteria offers some assurance that particular pathogens are not present at unacceptably high concentrations, but does not guarantee "absence" of those pathogens.

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs introduces two different types of criteria; Food Safety Criteria and Process Hygiene Criteria. A Food Safety Criterion is defined in the EU-legislation as a criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market. A Process hygiene criterion is defined as a criterion indicating the acceptable functioning of the production process. Such a criterion is not applicable to products placed on the market. It sets an indicative contamination value above which corrective actions are required in order to maintain the hygiene of the process in compliance with food law. A process hygiene criterium communicates the expected outcome of a process as end manufacturing or end- product criteria. They define the expected final outcome of the processes, but they neither characterize nor differentiate between the processes themselves³³.

An advantage of establishing food safety criteria for pathogenic microorganisms is that harmonised standards on the acceptability of food are provided for both authorities and industry within the EU and for products imported from third countries. Food safety criteria will impact the entire food chain, as they are set for products placed on the market. Risk of recalls and the economic loss as well as loss of consumer confidence will be a strong motivation to meet the criteria. Therefore food safety criteria are assumed to have an effect on food safety and public health where there is an actual or perceived risk. However, it is not possible to evaluate the extent of public health protection provided by a specific food safety criterion. Microbiological testing alone may convey a false sense of security due to the statistical limitation of sampling plans, particularly in the cases where the hazard presents an unacceptable risk at low concentrations and/or low and variable prevalences. Food safety is a result of several factors.

It is recommended that the goal for risk management is established before evaluating possible control options, including the establishment of microbiological criteria and their purpose.

7.2. Specific criteria/limits for viruses in food.

PCR-based detection methods exist for NoV, HAV and HEV in a range of foodstuffs and environmental matrices and can be used by food business operators to evaluate whether viral contamination occurs in their food supply chains, and to inform control options (e.g. HACCP plans).

NoV can be frequently detected in bivalve molluscs. There are no “safe limits” (threshold for infectivity) for NoV detected by PCR, since molluscs carrying low viral loads have been associated with human outbreaks. Data are rare and quantification of NoV in shellfish implicated in outbreaks varies from hundred thousand RNA copies/g of oyster digestive tissues. However, some samples with less than hundred RNA copies/ g of oyster digestive tissues have been demonstrated to be responsible for human cases both in UK or France (Baker et al., 2011; Le Guyader et al., 2008; Lowther et al., 2010). The probability of becoming infected increases with the dose, as was observed in volunteer studies and during outbreaks. Also, a correlation has been found between the number of NoV genome copies in oysters, and the amount of self reported illness in a specific study in the UK (Lowther et al., 2010).

Regulation (EC) 2073/2005 indicates that criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. Furthermore, regulation (EC) No 853/2004 provides a possibility to lay down additional health standards for live bivalve molluscs including virus testing procedures, and virological standards. Assuming that quantitative data on viral load is available, it would be possible to establish criteria for NoV in bivalve molluscs, while considering the impact of a given criteria on the exposure of the consumer. To this respect, work is in progress in EFSA on a mandate on “*Norovirus in oysters: methods, limits and control options*”³⁴.

³³ Opinion of the Scientific Panel on biological hazards (BIOHAZ) on microbiological criteria and targets based on risk analysis. www.efsa.europa.eu/en/efsajournal/pub/462.htm

³⁴ www.efsa.europa.eu/en/request/requests.htm

Viruses can be detected in fresh produce, but prevalence studies are limited, and quantitative data on viral load is scarce making establishment of microbiological criteria for these food categories difficult. Although there are documented cases of derived illness, the relative contribution of fresh produce to the overall public health FBV risk has not been established.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

- Foodborne transmission has been documented for viruses belonging to at least 10 families, and the diseases associated with these infections range from mild diarrhoeal illness to severe disease (e.g. encephalitis). Asymptomatic infections also occur.
- NoV and HAV in bivalve molluscs, fresh produce and ready-to-eat foods are the most frequently recognised causes of foodborne illness among all virus / food commodity combinations. HEV is highly prevalent in pigs across Europe. Rare cases of foodborne infection have been reported, although the source of most of the endemic human cases remains unknown. For these reasons, the present opinion focuses on these virus/food commodity combinations. However, the potential for foodborne transmission for other emerging viruses with high public health impact should not be neglected.

Conclusions on ToR1. Biology, epidemiology, diagnosis and public health importance of foodborne viruses. Data needs to support a risk assessment.

- At community level, NoV infection is the most common cause of infectious human gastroenteritis. NoV is shed in huge quantities in the stool and vomit of infected persons, and oral exposure to only a few particles is sufficient to cause disease. Viral excretion may start before the onset of symptoms, or continue after clinical recovery. In addition, some infected people shedding viruses for longer periods may never show symptoms. Illness typically is mild and self-limiting, but may be more severe and even fatal in elderly and immuno-compromised individuals.
- HAV is the aetiological agent of the most common type of hepatitis worldwide. The virus is shed in high numbers in the faeces of both symptomatic and asymptomatic individuals. Even in symptomatic cases, virus shedding starts before the onset of symptoms. Infectivity is unknown but may be very high. HAV does not result in a chronic infection, but it may occasionally evolve to a fulminant hepatitis.
- In contrast to NoV and HAV, HEV has been identified also as a zoonosis. Although rare, its importance is increasingly recognised in the EU. Clinical disease in humans is mainly characterized by acute hepatitis with average worldwide case fatality ratios between 1 and 5%, which may be higher in pregnant women under certain circumstances. Case fatality ratios for the most common genotype variants circulating in Europe are not known. Other high risk groups like people with underlying liver disease and immuno-compromised persons may develop severe or chronic disease courses (as has been shown for genotype 1). The dose response relationship for HEV for humans, is unknown.
- In the EU, the major mode of transmission for NoV remains person-to-person (directly from the human reservoir).
- In the EU, the major mode of transmission for HAV is person-to-person (directly or indirectly from the human reservoir), mainly as a consequence of travelling to endemic regions, having risky sexual practices, or consuming contaminated water or food.

- Food may be contaminated by virus during all stages of the food supply chain, and transmission can occur by consumption of food contaminated during the production process (primary production, or during further processing), or contaminated by infected food handlers. Transmission of zoonotic viruses (e.g. HEV) can also occur by consumption of products of animal origin, although few cases are reported.
- Viruses do not multiply in foods, but may persist for extended periods of time as infectious particles in the environment, or in foods. Therefore viruses, if they contaminate a foodstuff will often remain infectious and may constitute a risk to the consumer.
- At the EU-level it is unknown how much disease caused by NoV can be attributed to foodborne spread. Studies in some countries suggest that this can be significant. The relative contribution of different sources (shellfish, fresh produce, food handler including asymptomatic shedders, food handling environment) to foodborne illness has not been determined. Current EU surveillance for foodborne NoV illness does not capture dispersed outbreaks very efficiently, and there is clear evidence of significant underreporting of foodborne NoV outbreaks.
- The background data from case reports of HAV is often insufficient to prove foodborne transmission, but occasional outbreaks have been documented. With the decreasing immunity to HAV in the EU population, the probability of outbreaks is increasing.
- The diagnosis of HEV infections in humans is not routinely done in most laboratories, and therefore, there is considerable under diagnosis of this infection.

Conclusions on ToR2. Possible control options and their anticipated impact to prevent or reduce the number of foodborne viral human infections.

- Effective control strategies for NoV and HAV need to focus on prevention of contamination. Such prevention will have to occur primarily at the pre-harvest level for some products (bivalve molluscs, fresh produce for raw consumption), at the harvest level (manual handling during picking fresh fruits and vegetables), and at the post-harvest phase for others (manual preparation of ready-to-eat foods).
- CODEX Guidelines on the application of general principles of food hygiene to control of viruses in food are under development. Two annexes to control HAV and NoV in bivalve molluscs (I), and fresh produce (II) are being prepared.
- Commission Regulation (EC) 2073/2005 lays down microbiological criteria for foods. However no specific criteria are set for viruses. In addition, there are no specific requirements laid down in legislation for the quality of water used in the food supply chain (in primary production), except for drinking water.
- Compliance with EU *E. coli* standards in relation to products placed on the market, as well as to categorisation of production areas does not ensure absence of viruses in bivalve molluscs. Bivalve mollusc depuration as currently performed (validated according to *E. coli* criteria) is not a reliable control measure for viruses.
- Production of bivalve molluscs in the vicinity of discharges of human faecal pollution is a high risk practice for viral contamination.
- The Waters Framework Directive (2000/60/EC) does not contain any specific microbiological standards for shellfish, and therefore is unlikely to provide sufficient safeguards for prevention of virus contamination in bivalve mollusc harvesting areas.

- There are indications that relaying for long period in combination with depuration is capable of reducing virus levels
- There are currently no effective post-harvest control options, except sufficient heat treatment, to eliminate the public health risk from viral contamination of both bivalves and fresh produce. The effects of heat treatment, acidification, hydrostatic pressure processing, and reduced water activity on virus infectivity in foods are highly dependent on virus type and food matrix, including its physico-chemical characteristics.
- UV irradiation can be effective for the inactivation of viruses on surfaces for food preparation and for the inactivation of viruses in water and aerosols, but can not be considered an effective generic measure to reduce viral loads on food.
- Current practice with ante-mortem and post-mortem inspection of meat will not detect HEV infected animals at the time of slaughter, and thus virus may be present in liver or meat. Presently the only efficient known control option for HEV infection from consumption of meat or liver is sufficient heat treatment.

Conclusions on ToR3. Establishment of microbiological criteria (food safety criteria and process hygiene criteria) for viruses

- Microbiological criteria for HAV and NoV are useful for validation and verification of HACCP-based processes and procedures, and can be used to communicate to food business operators what is an acceptable or unacceptable viral load.
- PCR-based detection methods exist for NoV, HAV and HEV in a range of foodstuffs and environmental matrices. Harmonization and standardization is currently ongoing for NoV and HAV in shellfish, fresh produce, and food surfaces. Standard methods are expected in 2012.
- NoV can be frequently detected in bivalve molluscs. There are no “safe limits” (threshold for infectivity) for NoV detected by PCR, since molluscs carrying low viral loads have been associated with human outbreaks. However, the probability of becoming infected increases with the dose, as was observed in volunteer studies and during outbreaks. Also, a correlation has been found between the number of NoV genome copies in oysters, and the amount of self reported illness in a specific study in the UK.
- Regulation (EC) 2073/2005 indicates that criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. Furthermore, regulation (EC) No 853/2004 provides a possibility to lay down additional health standards for live bivalve molluscs including virus testing procedures, and virological standards. Assuming that quantitative data on viral load is available, it would be possible to establish criteria for NoV in bivalve molluscs, while considering the impact of a given criteria on the exposure of the consumer.
- Viruses can be detected in fresh produce, but prevalence studies are limited, and quantitative data on viral load is scarce making establishment of microbiological criteria for these food categories difficult. Although there are documented cases of derived illness, the relative contribution of fresh produce to the overall public health FBV risk has not been established.

RECOMMENDATIONS

Related to Control options

- It is recommended to focus on preventive measures to avoid viral contamination rather than trying to remove/inactivate these viruses from food.
- Introduction of microbiological criteria for viruses in bivalve molluscs, unless they are labelled “to be cooked before consumption”. Food business operators should validate their control options to meet the established virus criteria.
- Using an *E. coli* standard for monitoring and classification of bivalve mollusc production areas provides general information about the background level of faecal contamination, and should be retained. The regulatory standards and monitoring approaches could be refined to improve public health protection. Introduction of virus microbiological criteria for classification of high risk bivalve molluscs (to be consumed raw) production areas should be considered. A virus monitoring programme for compliance with these criteria should be risk based according to the findings of a sanitary survey.
- It is recommended that EU environmental legislation considers specific protection against faecal pollution to bivalve mollusc production areas.
- Control measures need to focus on avoiding faecal contamination in mollusc production areas as much as possible. Sanitary surveys would provide the necessary knowledge base. Preventative approaches could include: introduction of prohibition zones in the proximity of sewage discharges, more stringent *E. coli* standards for class B classification areas, and the use of pollution alert procedures.
- Postharvest treatments should be validated for virucidal activity (e.g. using HAV as a model) to ensure that the treatments are effective, and can be applied consistently prior to implementation in the food production chain.
- Further training of food handlers about hygiene requirements and about specific viral contamination of foods and food preparation environments is recommended in order to reduce the risk of contamination of ready-to eat foods.
- High risk groups (people with underlying liver disease, immuno-compromised persons and pregnant women) should be discouraged from eating meat and liver derived from wild boars and domestic pigs without proper cooking for prevention of hepatitis E.

Related to data needs

- Routine harmonised surveillance of NoV, and of virus occurrence in food commodities including molecular typing is recommended to aid source attribution studies. For HEV and HAV, notification and systematic strain typing of viruses in humans and in animals (HEV) and food commodities (HAV) is needed to get a better understanding of sources of virus.
- Population-level estimates of incidence, risk factors and clinical impact of NoV, HAV, and HEV in humans in general, and in specific risk groups (e.g. immuno-compromised individuals, elderly) are needed to determine the burden of disease, including foodborne illness.
- Studies are needed to determine the importance of presymptomatic, postsymptomatic, and asymptomatic shedding of NoV and HAV as sources of foodborne human infection.
- In order to quantify the efficacy of specific control options, it is necessary to build a quantitative risk assessment framework. This should be done for specific priority virus-commodity combinations, including consideration of the target population.

- Data needs for QMRA of FBV include: consumer habits, virus contamination levels in food and other reservoirs, virus transfer rates, natural persistence on/in foods (at the pre-harvest and post-harvest levels), and human dose-response relations. These data should be collected based on specific targeted studies, including sampling strategies.
- More studies are needed on the relation between detection of virus genomic copies by PCR in food and probability of causing disease. For this purpose, a guidance for outbreak investigation for FBV-related outbreaks could be drawn up to generate the type of data needed for QMRA.
- Studies are needed to determine the importance of foodborne transmission pathways for HEV. This will help the establishment of control options.

REFERENCES

- Abad FX, Pinto RM and Bosch A, 1994a. Survival of enteric viruses on environmental fomites. *Appl Environ Microbiol*, 60, 3704-3710.
- Abad FX, Pinto RM, Diez JM and Bosch A, 1994b. Disinfection of human enteric viruses in water by copper and silver in combination with low levels of chlorine. *Appl Environ Microbiol*, 60, 2377-2383.
- Abad FX, Pinto RM, Gajardo R and Bosch A, 1997. Viruses in mussels: Public health implications and depuration. *Journal of Food Protection*, 60, 677-681.
- AFSA (Agence française de sécurité sanitaire des aliments), 2009. Synthèse de l'étude individuelle nationale des consommations alimentaires 2 (INCA 2) 2006-2007. 1-44.
- Aggarwal R and Jameel S, 2008. Hepatitis E vaccine. *Hepatology*, 2, 308-315.
- Aggarwal R and Naik S, 2009. Epidemiology of hepatitis E: current status. *J Gastroenterol Hepatol*, 24, 1484-1493.
- Akriviadis EA and Redeker AG, 1989. Fulminant hepatitis A in intravenous drug users with chronic liver disease. *Ann Intern Med*, 110, 838-839.
- Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A and Andersson B, 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA*, 102, 12891-12896.
- Allwood PB, Malik YS, Maherchandani S, Vought K, Johnson LA, Braymen C, Hedberg CW and Goyal SM, 2004. Occurrence of *Escherichia coli*, noroviruses, and F-specific coliphages in fresh market-ready produce. *J Food Prot*, 67, 2387-2390.
- Anderson AD, Garrett VD, Sobel J, Monroe SS, Fankhauser RL, Schwab KJ, Bresee JS, Mead PS, Higgins C, Campana J and Glass RI, 2001. Multistate outbreak of Norwalk-like virus gastroenteritis associated with a common caterer. *American Journal of Epidemiology*, 154, 1013-1019.
- Aragones L, Bosch A and Pinto RM, 2008. Hepatitis A virus mutant spectra under the selective pressure of monoclonal antibodies: codon usage constraints limit capsid variability. *J Virol*, 82, 1688-1700.
- Aragones L, Guix S, Ribes E, Bosch A and Pinto RM, 2010. Fine-tuning translation kinetics selection as the driving force of codon usage bias in the hepatitis A virus capsid. *PLoS Pathog*, 6, e1000797.
- Atmar RL, Neill FH, Romalde JL, Le Guyader F, Woodley CM, Metcalf TG and Estes MK, 1995. Detection of Norwalk virus and hepatitis A virus in shellfish tissues with the PCR. *Appl Environ Microbiol*, 61, 3014-3018.
- Atmar RL, Opekun AR, Gilger MA, Estes MK, Crawford SE, Neill FH and Graham DY, 2008. Norwalk virus shedding after experimental human infection. *Emerg Infect Dis*, 14, 1553-1557.
- Bachlein C and Grummer B, 2010. Hepatitis E - a new zoonotic disease in Germany? *Berl Munch Tierarztl Wochenschr*, 123, 198-204.
- Baert L, Uyttendaele M and Debevere J, 2008a. Evaluation of viral extraction methods on a broad range of Ready-To-Eat foods with conventional and real-time RT-PCR for Norovirus GII detection. *Int J Food Microbiol*, 123, 101-108.
- Baert L, Uyttendaele M, Van Coillie E and Debevere J, 2008b. The reduction of murine norovirus 1, *B. fragilis* HSP40 infecting phage B40-8 and *E. coli* after a mild thermal pasteurization process of raspberry puree. *Food Microbiology*, 25, 871-874.
- Baert L, Uyttendaele M, Vermeersch M, Van Coillie E and Debevere J, 2008c. Survival and transfer of murine norovirus 1, a surrogate for human noroviruses, during the production process of deep-frozen onions and spinach. *J Food Prot*, 71, 1590-1597.

- Baert L, Vandekinderen I, Devlieghere F, Van Coillie E, Debevere J and Uyttendaele M, 2009. Efficacy of sodium hypochlorite and peroxyacetic acid to reduce murine norovirus 1, B40-8, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 on shredded iceberg lettuce and in residual wash water. *J Food Prot*, 72, 1047-1054.
- Baker K, Morris J, McCarthy N, Saldana L, Lowther J, Collinson A and Young M, 2011. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. *J Public Health (Oxf)*, 33:205-211,
- Baroudy BM, Ticehurst JR, Miele TA, Maizel JV, Jr., Purcell RH and Feinstone SM, 1985. Sequence analysis of hepatitis A virus cDNA coding for capsid proteins and RNA polymerase. *Proc Natl Acad Sci U S A*, 82, 2143-2147.
- Barzaga BN, 2000. Hepatitis A shifting epidemiology in South-East Asia and China. *Vaccine*, 18 Suppl 1, S61-64.
- Becker W and Pearson M 1998. Riksmaten 1997-98. Kostvanor och näringsintag I Sverige. Metod-och resultatanalysis. 1-201. available at: www.slv.se/upload/dokument/rapporter/kostundersokningar/riksmat.pdf.
- Beersma MF, Schutten M, Vennema H, Hartwig NG, Mes TH, Osterhaus AD, van Doornum GJ and Koopmans M, 2009. Norovirus in a Dutch tertiary care hospital (2002-2007): frequent nosocomial transmission and dominance of GIIB strains in young children. *J Hosp Infect*, 71, 199-205.
- Beuchat LR (Food safety unit World Health Organization), 1998. Surface decontamination of fruits and vegetables eaten raw: a review. WHO/FSF/FOS/98.2.
- Beuret C, Baumgartner A and Schluep J, 2003. Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. *Appl Environ Microbiol*, 69, 2292-2297.
- Bidawid S, Farber J, Sattar SA and Hayward S, 2000a. Heat inactivation of hepatitis A virus in dairy foods. *Journal of Food Protection*, 63, 522-528.
- Bidawid S, Farber JM and Sattar SA, 2000b. Contamination of foods by food handlers: experiments on hepatitis A virus transfer to food and its interruption. *Appl Environ Microbiol*, 66, 2759-2763.
- Bidawid S, Farber JM, Sattar SA and Hayward S, 2000c. Heat inactivation of hepatitis A virus in dairy foods. *J Food Prot*, 63, 522-528.
- Bidawid S, Malik N, Adegbinrin O, Sattar SA and Farber JM, 2004. Norovirus cross-contamination during food handling and interruption of virus transfer by hand antisepsis: Experiments with feline calicivirus as a surrogate. *Journal of Food Protection*, 67, 103-109.
- Borman AM, Kirchweiger R, Ziegler E, Rhoads RE, Skern T and Kean KM, 1997. eIF4G and its proteolytic cleavage products: effect on initiation of protein synthesis from capped, uncapped, and IRES-containing mRNAs. *RNA*, 3, 186-196.
- Bosch A, 1995. The survival of enteric viruses in the water environment. *Microbiologia*, 11, 393-396.
- Bosch A, Lucena F, Diez JM, Gajardo R and Blasi M, 1991. Waterborne Viruses Associated with Hepatitis Outbreak. *Journal of the American Water Works Association*, 83, 80-83.
- Bosch A and Pinto RM, 2010. Hepatitis A and E Viruses. In: *Genomes of Food- and Water-Borne Pathogens*. P Fratamico, S Kathariou, Y Liu. ASM Press, Washington DC,
- Bouwknegt M, Lodder-Verschoor F, van der Poel WH, Rutjes SA and de Roda Husman AM, 2007. Hepatitis E virus RNA in commercial porcine livers in The Netherlands. *J Food Prot*, 70, 2889-2895.
- Bouwknegt M, Rutjes SA, Reusken CB, Stockhofe-Zurwieden N, Frankena K, de Jong MC, de Roda Husman AM and Poel WH, 2009. The course of hepatitis E virus infection in pigs after contact-infection and intravenous inoculation. *BMC Vet Res*, 5, 7.

- Bower WA, Nainan OV, Han X and Margolis HS, 2000. Duration of viremia in hepatitis A virus infection. *J Infect Dis*, 182, 12-17.
- Boxman I, Dijkman R, Verhoef L, Maat A, van Dijk G, Vennema H and Koopmans M, 2009. Norovirus on swabs taken from hands illustrate route of transmission: a case study. *J Food Prot*, 72, 1753-1755.
- Boxman I, Verhoef L, Dijkman R, Hagele G, Te Loeke NA and Koopmans M, 2011. Year-round prevalence of Norovirus in the environment of catering companies without a recently reported outbreak of gastroenteritis. *Appl Environ Microbiol* 77:2968-2974.
- Boxman IL, Tilburg JJ, Te Loeke NA, Vennema H, Jonker K, de Boer E and Koopmans M, 2006. Detection of noroviruses in shellfish in the Netherlands. *Int J Food Microbiol*, 108, 391-396.
- Boxman ILA, Tilburg JJHC, Loeke NAJM, Vennema H, de Boer E and Koopmans M, 2007. An efficient and rapid method for recovery of norovirus from food associated with outbreaks of gastroenteritis. *Journal of Food Protection*, 70, 504-508.
- Briese T, Paweska JT, McMullan LK, Hutchison SK, Street C, Palacios G, Khristova ML, Weyer J, Swanepoel R, Egholm M, Nichol ST and Lipkin WI, 2009. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. *PLoS Pathog*, 5, e1000455.
- Buckow R, Isbarn S, Knorr D, Heinz V and Lehmacher A, 2008. Predictive model for inactivation of feline calicivirus, a norovirus surrogate, by heat and high hydrostatic pressure. *Applied and Environmental Microbiology*, 74, 1030-1038.
- Butot S, Putallaz T, Croquet C, Lamothe G, Meyer R, Joosten H and Sanchez G, 2007a. Attachment of enteric viruses to bottles. *Appl Environ Microbiol*, 73, 5104-5110.
- Butot S, Putallaz T and Sanchez G, 2007b. Procedure for rapid concentration and detection of enteric viruses from berries and vegetables. *Appl Environ Microbiol*, 73, 186-192.
- Butot S, Putallaz T and Sanchez G, 2008. Effects of sanitation, freezing and frozen storage on enteric viruses in berries and herbs. *Int J Food Microbiol*, 126, 30-35.
- Calci KR, Meade GK, Tezloff RC and Kingsley DH, 2005. High-pressure inactivation of hepatitis A virus within oysters. *Applied and Environmental Microbiology*, 71, 339-343.
- Callahan KM, Taylor DJ and Sobsey MD, 1995. Comparative survival of hepatitis A virus, poliovirus and indicator viruses in geographically diverse seawaters. *Water Sci Technol*, 31, 189-193.
- Cannon JL, Papafragkou E, Park GW, Osborne J, Jaykus LA and Vinje J, 2006. Surrogates for the study of norovirus stability and inactivation in the environment: a comparison of murine norovirus and feline calicivirus. *J Food Prot*, 69, 2761-2765.
- Carrique-Mas J, Andersson Y, Petersen B, Hedlund KO, Sjogren N and Giesecke J, 2003. A norwalk-like virus waterborne community outbreak in a Swedish village during peak holiday season. *Epidemiol Infect*, 131, 737-744.
- Carter MJ, 2005. Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology*, 98, 1354-1380.
- Casteel MJ, Schmidt CE and Sobsey MD, 2008. Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. *International Journal of Food Microbiology*, 125, 267-273.
- CDC, 1997. Hepatitis A associated with consumption of frozen strawberries--Michigan, March 1997. *MMWR Morb Mortal Wkly Rep*, 46, 288, 295.
- Chan MC, Sung JJ, Lam RK, Chan PK, Lee NL, Lai RW and Leung WK, 2006. Fecal viral load and norovirus-associated gastroenteritis. *Emerg Infect Dis*, 12, 1278-1280.
- Chandra V, Taneja S, Kalia M and Jameel S, 2008. Molecular biology and pathogenesis of hepatitis E virus. *J Biosci*, 33, 451-464.

- Chauhan A, Jameel S, Dilawari JB, Chawla YK, Kaur U and Ganguly NK, 1993. Hepatitis E virus transmission to a volunteer. *Lancet*, 341, 149-150.
- Cheesbrough JS, Green J, Gallimore CI, Wright PA and Brown DW, 2000. Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiol Infect*, 125, 93-98.
- Cheesebrough JS, Barkess-Jones L and Brown DW, 1997. Possible prolonged environmental survival of small round structured viruses. *Journal of Hospital Infection*, 35, 324-326.
- Chen HQ, Hoover DG and Kingsley DH, 2005. Temperature and treatment time influence high hydrostatic pressure inactivation of feline calicivirus, a norovirus surrogate. *Journal of Food Protection*, 68, 2389-2394.
- Cheng PK, Wong DK, Chung TW and Lim WW, 2005. Norovirus contamination found in oysters worldwide. *J Med Virol*, 76, 593-597.
- Ching KZ, Nakano T, Chapman LE, Demby A and Robertson BH, 2002. Genetic characterization of wild-type genotype VII hepatitis A virus. *J Gen Virol*, 83, 53-60.
- Chironna M, Germinario C, De Medici D, Fiore A, Di Pasquale S, Quarto M and Barbuti S, 2002. Detection of hepatitis A virus in mussels from different sources marketed in Puglia region (South Italy). *Int J Food Microbiol*, 75, 11-18.
- Chironna M, Lopalco P, Prato R, Germinario C, Barbuti S and Quarto M, 2004. Outbreak of infection with hepatitis a virus (HAV) associated with a foodhandler and confirmed by sequence analysis reveals a new HAV genotype IB variant. *Journal of Clinical Microbiology*, 42, 2825-2828.
- Cianciara J, 2000. Hepatitis A shifting epidemiology in Poland and Eastern Europe. *Vaccine*, 18 Suppl 1, S68-70.
- Clover DO, 1985. Vehicular transmission of hepatitis A. *Public Health Rev*, 13, 235-292.
- Clover DO, Ellender RD and Sobsey MD, 1983. Methods to detect viruses in food: testing and interpretation of results. *J Food Prot*, 46, 345-357.
- Cohen JI, Rosenblum B, Ticehurst JR, Daemer RJ, Feinstone SM and Purcell RH, 1987. Complete nucleotide sequence of an attenuated hepatitis A virus: comparison with wild-type virus. *Proc Natl Acad Sci USA*, 84, 2497-2501.
- Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, Heyries L, Raoult D and Gerolami R, 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis*, 202, 825-834.
- Conaty S, Bird P, Bell G, Kraa E, Grohmann G and McAnulty JM, 2000. Hepatitis A in New South Wales, Australia from consumption of oysters: the first reported outbreak. *Epidemiol Infect*, 124, 121-130.
- Costa-Mattioli M, Cristina J, Romero H, Perez-Bercof R, Casane D, Colina R, Garcia L, Vega I, Glikman G, Romanowsky V, Castello A, Nicand E, Gassin M, Billaudel S and Ferre V, 2002. Molecular evolution of hepatitis A virus: a new classification based on the complete VP1 protein. *J Virol*, 76, 9516-9525.
- Costafreda MI, Bosch A and Pinto RM, 2006. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Appl Environ Microbiol*, 72, 3846-3855.
- Costantini V, Loisy F, Joens L, Le Guyader FS and Saif LJ, 2006. Human and animal enteric caliciviruses in oysters from different coastal regions of the United States. *Appl Environ Microbiol*, 72, 1800-1809.
- Crance JM, Gantzer C, Schwartzbrod L and Deloince R, 1998. Effect of temperature on the survival of hepatitis A virus and its capsid antigen in synthetic seawater. *Env Tox Water Quality*, 13, 89-92.

- Croci L, Ciccozzi M, de Medici D, di Pasquale S, Fiore A, Mele A and Todi L, 1999. Inactivation of hepatitis A virus in heat-treated mussels. *Journal of Applied Microbiology*, 87, 884-888.
- Croci L, De Medici D, Scalfaro C, Fiore A, Divizia M, Donia D, Cosentino AM, Moretti P and Costantini G, 2000. Determination of enteroviruses, hepatitis A virus, bacteriophages and *Escherichia coli* in Adriatic Sea mussels. *J Appl Microbiol*, 88, 293-298.
- Croci L, De Medici D, Scalfaro C, Fiore A and Toti L, 2002. The survival of hepatitis A virus in fresh produce. *Int J Food Microbiol*, 73, 29-34.
- Croci L, Dubois E, Cook N, de Medici D, Schultz AC, China B, Rutjes S, Hoorfar J and van der Poel WHM, 2008. Current methods for extraction and concentration of enteric viruses from fresh fruit and vegetables: towards international standards. *Food Anal. Methods*, 1, 73-84.
- Dagan R, Leventhal A, Anis E, Slater P, Ashur Y and Shouval D, 2005. Incidence of hepatitis A in Israel following universal immunization of toddlers. *JAMA*, 294, 202-210.
- Dawson DJ, Paish A, Staffell LM, Seymour IJ and Appleton H, 2005. Survival of viruses on fresh produce, using MS2 as a surrogate for norovirus. *Journal of Applied Microbiology*, 98, 203-209.
- de Bruin E, Duizer E, Vennema H and Koopmans MP, 2006. Diagnosis of Norovirus outbreaks by commercial ELISA or RT-PCR. *J Virol Methods*, 137, 259-264.
- De Medici D, Croci L, Di Pasquale S, Fiore A and Toti L, 2001. Detecting the presence of infectious hepatitis A virus in molluscs positive to RT-nested-PCR. *Lett Appl Microbiol*, 33, 362-366.
- de Roda Husman AM, Bijkerk P, Lodder W, van den Berg H, Pribil W, Cabaj A, Gehringer P, Sommer R and Duizer E, 2004. Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (Gamma) radiation. *Applied and Environmental Microbiology*, 70, 5089-5093.
- De Vriese S, Huybrecht I, Moreau M, De Henauw S, De Backer G, Kornlitzer M, Leveque A and Van Oyen H, 2005. The Belgian food consumption survey: aim, design and methods. *Arch Public Health*, 63, 1-16.
- de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J and van Leusden F, 2001. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. *American Journal of Epidemiology*, 154, 666-674.
- de Wit MA, Koopmans MP and van Duynhoven YT, 2003. Risk factors for norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. *Emerg Infect Dis*, 9, 1563-1570.
- de Wit MA, Widdowson MA, Vennema H, de Bruin E, Fernandes T and Koopmans M, 2007. Large outbreak of norovirus: the baker who should have known better *Journal of Infection*, 55, 188-193.
- Deboosere N, Legeay O, Caudrelier Y and Lange M, 2004. Modelling effect of physical and chemical parameters on heat inactivation kinetics of hepatitis A virus in a fruit model system. *Int J Food Microbiol*, 93, 73-85.
- Dentinger CM, Bower WA, Nainan OV, Cotter SM, Myers G, Dubusky LM, Fowler S, Salehi ED and Bell BP, 2001. An outbreak of hepatitis A associated with green onions. *J Infect Dis*, 183, 1273-1276.
- Depaola A, Jones JL, Woods J, Burkhardt W, 3rd, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D and Nabe K, 2010. Bacterial and Viral Pathogens in Live Oysters: U.S. Market Survey 2007. *Appl Environ Microbiol*,
- Di Girolamo R, Liston J and Matches JR, 1970. Survival of virus in chilled, frozen, and processed oysters. *Applied Microbiology*, 20, 58-63.
- Dolin R, DuPont HL, Wyatt RG, Hornick R, Buscho RF, Chanock RM, Blacklow NR and Kasel JA, 1972. Biological properties of Norwalk agent of acute infectious nonbacterial gastroenteritis. *Proceedings of the Society for Experimental Biology and Medicine*, 140, 578-583.

- Domingo E, Martin V, Perales C, Grande-Perez A, Garcia-Arriaza J and Arias A, 2006. Viruses as quasispecies: biological implications. *Curr Top Microbiol Immunol*, 299, 51-82.
- Dominguez A, Oviedo M, Carmona G, Batalla J, Bruguera M, Salleras L and Plasencia A, 2008. Impact and effectiveness of a mass hepatitis A vaccination programme of preadolescents seven years after introduction. *Vaccine*, 26, 1737-1741.
- Dore B, Keaveney S, Flannery J and Rajko-Nenow P, 2010. Management of health risks associated with oysters harvested from a norovirus contaminated area, Ireland, February-March 2010. *Euro Surveill*, 15, pii/19567.
- Dore WJ, Henshilwood K and Lees DN, 2000. Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. *Appl Environ Microbiol*, 66, 1280-1285.
- Dore WJ and Lees DN, 1995. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. *Appl Environ Microbiol*, 61, 2830-2834.
- Doultree JC, Druce JD, Birch CJ, Bowden DS and Marshall JA, 1999. Inactivation of feline calicivirus, a Norwalk virus surrogate. *Journal of Hospital Infection*, 41, 51-57.
- Doyle A, Barataud D, Gallay A, Thiolet JM, Le Guyaguer S, Kohli E and Vaillant V, 2004. Norovirus foodborne outbreaks associated with the consumption of oysters from the Etang de Thau, France, December 2002. *Euro Surveill*, 9, 24-26.
- Drexler JF, Gloza-Rausch F, Glende J, Corman VM, Muth D, Goettsche M, Seebens A, Niedrig M, Pfefferle S, Yordanov S, Zhelyazkov L, Hermanns U, Vallo P, Lukashev A, Muller MA, Deng H, Herrler G and Drosten C, 2010. Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. *J Virol*, 84, 11336-11349.
- Dubois E, Hennechart C, Deboosere N, Merle G, Legeay O, Burger C, Le Calve M, Lombard B, Ferre V and Traore O, 2006. Intra-laboratory validation of a concentration method adapted for the enumeration of infectious F-specific RNA coliphage, enterovirus, and hepatitis A virus from inoculated leaves of salad vegetables. *Int J Food Microbiol*, 108, 164-171.
- Dubois E, Hennechart C, Merle G, Burger C, Hmila N, Ruelle S, Perelle S and Ferre V, 2007. Detection and quantification by real-time RT-PCR of hepatitis A virus from inoculated tap waters, salad vegetables, and soft fruits: characterization of the method performances. *Int J Food Microbiol*, 117, 141-149.
- Duizer E, Bijkerk P, Rockx B, de Groot A, Twisk F and Koopmans M, 2004. Inactivation of caliciviruses. *Applied and Environmental Microbiology*, 70, 4538-4543.
- Duizer E and Koopmans M, 2008. Emerging food-borne viral diseases. In: *Food-borne viruses: Progress and Challenges*. Eds: M Koopmans, DO Cliver, A Bosch. ASM Press, Washington DC,
- Duizer E, Kroneman A, Siebenga J, Verhoef L, Vennema H and Koopmans M, 2008. Typing database for noroviruses. *Euro Surveill*, 13.
- Elamri DE, Aouni M, Parnaudeau S and Le Guyader FS, 2006. Detection of human enteric viruses in shellfish collected in Tunisia. *Lett Appl Microbiol*, 43, 399-404.
- Elmadfa I, Freisling H, Nowak V and Hofstädter D 2009. *Austrian Nutrition Report 2008 (Österreichischer Ernährungsbericht 2008)*. 1st edition.
- Emerson SU, Arankalle VA and Purcell RH, 2005. Thermal stability of hepatitis E virus. *J Infect Dis*, 192, 930-933.
- Emerson SU, Huang YK, Nguyen H, Brockington A, Govindarajan S, St Claire M, Shapiro M and Purcell RH, 2002. Identification of VP1/2A and 2C as virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. *J Virol*, 76, 8551-8559.

- Enriquez CE, Hurst CJ and Gerba CP, 1995. Survival of enteric adenoviruses 40 and 41 in tap, sea, and waste water. *Water Res*, 29, 2548-2553.
- Ethelberg S, Lisby M, Bottiger B, Schultz AC, Villif A, Jensen T, Olsen KE, Scheutz F, Kjelson C and Muller L, 2010. Outbreaks of gastroenteritis linked to lettuce, Denmark, January 2010. *Euro Surveill*, 15.
- Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI and Westmoreland D, 2002. An outbreak of viral gastroenteritis following environmental contamination at a concert hall. *Epidemiol Infect*, 129, 355-360.
- Falkenhorst G, Krusell L, Lisby M, Madsen SB, Bottiger B and Molbak K, 2005. Imported frozen raspberries cause a series of norovirus outbreaks in Denmark, 2005. *Euro Surveill*, 10.
- Fankhauser RL, Monroe SS, Noel JS, Humphrey CD, Bresee JS, Parashar UD, Ando T and Glass RI, 2002. Epidemiologic and molecular trends of Norwalk-like viruses associated with outbreaks of gastroenteritis in the United States. *J Infect Dis*, 186, 1-7.
- Fankhauser RL, Noel JS, Monroe SS, Ando T and Glass RI, 1988. Molecular epidemiology of Norwalk-like viruses in outbreaks of gastroenteritis in the United States. *J Infect Dis*, 178, 1571-1578.
- Farkas T, Berke T, Reuter G, Szûcs G, Matson DO and Jiang X, 2002. Molecular detection and sequence analysis of human caliciviruses from acute gastroenteritis outbreaks in Hungary. *J Med Virol*, 67, 567-573.
- Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA 2005. *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*. New York, USA.
- Feagins AR, Opriessnig T, Guenette DK, Halbur PG and Meng XJ, 2007. Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. *J Gen Virol*, 88, 912-917.
- Feagins AR, Opriessnig T, Guenette DK, Halbur PG and Meng XJ, 2008. Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. *Int J Food Microbiol*, 123, 32-37.
- Fino VR and Kniel KE, 2008. UV light inactivation of hepatitis A virus, aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *Journal of Food Protection*, 71, 908-913.
- Flannery J, Keaveney S and Dore W, 2009. Use of FRNA bacteriophages to indicate the risk of norovirus contamination in Irish oysters. *J Food Prot*, 72, 2358-2362.
- Formiga-Cruz M, Allard AK, Conden-Hansson AC, Henshilwood K, Hernroth BE, Jofre J, Lees DN, Lucena F, Papapetropoulou M, Rangdale RE, Tsibouxi A, Vantarakis A and Girones R, 2003. Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas. *Appl Environ Microbiol*, 69, 1556-1563.
- Formiga-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson AC, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD and Girones R, 2002. Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden, and the United Kingdom. *Appl Environ Microbiol*, 68, 5990-5998.
- Franco E and Vitiello G, 2003. Vaccination strategies against hepatitis A in southern Europe. *Vaccine*, 21, 696-697.
- Fretz R, Svoboda P, Schorr D, Tanner M and Baumgartner A, 2005. Risk factors for infections with Norovirus gastrointestinal illness in Switzerland. *Eur J Clin Microbiol Infect Dis*, 24, 256-261.
- Fujiwara K, Yokosuka O, Ehata T, Saisho H, Saotome N, Suzuki K, Okita K, Kiyosawa K and Omata M, 2002. Association between severity of type A hepatitis and nucleotide variations in the 5' non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut*, 51, 82-88.

- Fujiwara K, Yokosuka O, Fukai K, Imazeki F, Saisho H and Omata M, 2001. Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. *J Hepatol*, 35, 112-119.
- Fujiwara K, Yokosuka O, Imazeki F, Saisho H, Saotome N, Suzuki K, Okita K, Tanaka E and Omata M, 2003. Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. *Hepatol Res*, 25, 124-134.
- Gabrieli R, Macaluso A, Lanni L, Saccares S, Di Giamberardino F, Cencioni B, Petrinca AR and Divizia M, 2007. Enteric viruses in molluscan shellfish. *New Microbiol*, 30, 471-475.
- Gabrieli R, Sanchez G, Macaluso A, Cenko F, Bino S, Palombi L, Buonomo E, Pinto RM, Bosch A and Divizia M, 2004. Hepatitis in Albanian children: molecular analysis of hepatitis A virus isolates. *J Med Virol*, 72, 533-537.
- Gallimore CI, Barreiros MA, Brown DW, Nascimento JP and Leite JP, 2004a. Noroviruses associated with acute gastroenteritis in a children's day care facility in Rio de Janeiro, Brazil. *Braz J Med Biol Res*, 37, 321-326.
- Gallimore CI, Green J, Lewis D, Richards AF, Lopman BA, Hale AD, Eglin R, Gray JJ and Brown DW, 2004b. Diversity of noroviruses cocirculating in the north of England from 1998 to 2001. *J Clin Microbiol*, 42, 1396-1401.
- Gallimore CI, Pipkin C, Shrimpton H, Green AD, Pickford Y, McCartney C, Sutherland G, Brown DW and Gray JJ, 2005. Detection of multiple enteric virus strains within a foodborne outbreak of gastroenteritis: an indication of the source of contamination. *Epidemiol Infect*, 133, 41-47.
- Germinario C, Luigi Lopalco P, Chicanna M and Da Villa G, 2000. From hepatitis B to hepatitis A and B prevention: the puglia (Italy) experience. *Vaccine*, 18, 3326.
- Gerolami R, Moal V and Colson P, 2008. Chronic hepatitis E with cirrhosis in a kidney-transplant recipient. *N Engl J Med*, 358, 859-860.
- Girish R, Broor S, Dar L and Ghosh D, 2002. Foodborne outbreak caused by a Norwalk-like virus in India. *J Med Virol*, 67, 603-607.
- Glikson M, Galun E, Oren R, Tur-Kaspa R and Shouval D, 1992. Relapsing hepatitis A. Review of 14 cases and literature survey. *Medicine (Baltimore)*, 71, 14-23.
- Godoy P, Izcarra J, Bartolome R, Bach P, Escobar A, Pal M, Codina G, Torres J and Dominguez A, 2005. Outbreak of food-borne Norovirus associated with the consumption of sandwiches. *Med Clin (Barc)*, 124, 161-164.
- Gray JJ, Kohli E, Ruggeri FM, Vennema H, Sanchez-Fauquier A, Schreier E, Gallimore CI, Iturriza-Gomara M, Giraudon H, Pothier P, Di Bartolo I, Inglese N, de Bruin E, van der Veer B, Moreno S, Montero V, de Llano MC, Hohne M and Diedrich SM, 2007. European multicenter evaluation of commercial enzyme immunoassays for detecting norovirus antigen in fecal samples. *Clin Vaccine Immunol*, 14, 1349-1355.
- Green J, Vinje J, Gallimore CI, Koopmans M, Hale A, Brown DW, Clegg JC and Chamberlain J, 2000. Capsid protein diversity among Norwalk-like viruses. *Virus Genes*, 20, 227-236.
- Greening GE, Lake R, Hudson JA, Cressey P and Nortje G 2003. Risk profile: Norwalk-like virus in mollusca (raw). Client Report FW0312 prepared for NZ Food Safety Authority.
- Greig JD, Todd EC, Bartleson CA and Michaels BS, 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 1. Description of the problem, methods, and agents involved. *J Food Prot*, 70, 1752-1761.
- Grove SF, Forsyth S, Wan J, Coventry J, Cole M, Stewart CM, Lewis T, Ross T and Lee A, 2008. Inactivation of hepatitis A virus, poliovirus and a norovirus surrogate by high pressure processing. *Innovative Food Science & Emerging Technologies*, 206-210.

- Guan DS, Kniel KE, Calci KR, Hicks DT, Pivarnik LF and Hoover DG, 2006. Response of four types of coliphages to high hydrostatic pressure. *Food Microbiology*, 23, 546-551.
- Guevremont E, Brassard J, Houde A, Simard C and Trottier YL, 2006. Development of an extraction and concentration procedure and comparison of RT-PCR primer systems for the detection of hepatitis A virus and norovirus GII in green onions. *J Virol Methods*, 134, 130-135.
- Gulati BR, Allwood PB, Hedberg CW and Goyal SM, 2001. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. *Journal of Food Protection*, 64, 1430-1434.
- Gust ID, 1992. A vaccine against hepatitis A-at last. *Med J Aust*, 157, 345-346.
- Gyarmati P, Mohammed N, Norder H, Blomberg J, Belak S and Widen F, 2007. Universal detection of hepatitis E virus by two real-time PCR assays: TaqMan and Primer-Probe Energy Transfer. *J Virol Methods*, 146, 226-235.
- Haagsma EB, van den Berg AP, Porte RJ, Benne CA, Vennema H, Reimerink JH and Koopmans MP, 2008. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl*, 14, 547-553.
- Haas CN, Rose JB, Gerba CP and Regli S, 1993. Risk assessment of virus in drinking water. *Risk Anal*, 13, 545-552.
- Halliday ML, Kang LY, Zhou TK, Hu MD, Pan QC, Fu TY, Huang YS and Hu SL, 1991. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis*, 164, 852-859.
- Hamilton AJ, Stagnitti F, Premier R, Boland AM and Hale G, 2006. Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Appl Environ Microbiol*, 72, 3284-3290.
- Hernroth BE, Conden-Hansson AC, Rehnstam-Holm AS, Girones R and Allard AK, 2002. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the first Scandinavian report. *Appl Environ Microbiol*, 68, 4523-4533.
- Herremans M, Bakker J, Duizer E, Vennema H and Koopmans M, 2007. Use of serological assays for diagnosis of hepatitis E virus genotype 1 and 3 infections in a setting of low endemicity. *Clin Vaccine Immunol*, 14, 562-568.
- Hewitt J and Greening GE, 2006. Effect of heat treatment on hepatitis A virus and norovirus in New Zealand greenshell mussels (*Perna canaliculus*) by quantitative real-time reverse transcription PCR and cell culture. *J Food Prot*, 69, 2217-2223.
- Hewitt J, Rivera-Aban M and Greening GE, 2009. Evaluation of murine norovirus as a surrogate for human norovirus and hepatitis A virus in heat inactivation studies. *J Appl Microbiol*, 107, 65-71.
- Hirani ZM, DeCarolis JF, Adham SS and Jacangelo JG, 2010. Peak flux performance and microbial removal by selected membrane bioreactor systems. *Water Res*, 44, 2431-2440.
- Hollinger FB and Emerson SU, 2007. Hepatitis A virus. In: *Fields Virology*. Eds: DM Knipe, PM Howley. Lippincott Williams and Wilkins, Philadelphia, 911-947.
- Huang FF, Haqshenas G, Guenette DK, Halbur PG, Schommer SK, Pierson FW, Toth TE and Meng XJ, 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol*, 40, 1326-1332.
- Huang FF, Sun ZF, Emerson SU, Purcell RH, Shivaprasad HL, Pierson FW, Toth TE and Meng XJ, 2004. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *J Gen Virol*, 85, 1609-1618.
- Huang R, Li D, Wei S, Li Q, Yuan X, Geng L, Li X and Liu M, 1999. Cell culture of sporadic hepatitis E virus in China. *Clin Diagn Lab Immunol*, 6, 729-733.

- Huang YR, 2008. Application of electrolyzed water in the food industry. *Food Control*, 19, 329-345.
- Humphrey TJ and Martin K, 1993. Bacteriophages as models for virus removal from Pacific oysters (*Crassostrea gigas*) during re-laying. *Epidemiology and Infection*, 111, 325-335.
- Hutin YJ, Pool V, Cramer EH, Nainan OV, Weth J, Williams IT, Goldstein ST, Gensheimer KF, Bell BP, Shapiro CN, Alter MJ and Margolis HS, 1999. A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. *N Engl J Med*, 340, 595-602.
- Inoue J, Takahashi M, Mizuo H, Suzuki K, Aikawa T, Shimosegawa T and Okamoto H, 2009. Nucleotide substitutions of hepatitis E virus genomes associated with fulminant hepatitis and disease severity. *Tohoku J Exp Med*, 218, 279-284.
- Iritani N, Seto Y, Kubo H, Murakami T, Haruki K, Ayata M and Ogura H, 2003. Prevalence of Norwalk-like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. *J Clin Microbiol*, 41, 1756-1759.
- Jackson TA, 2002. Proteins involved in the function of picornavirus Internal Ribosome Entry sites. In: *Molecular biology of picornaviruses*. BLWE Semler. ASM Press, Washington DC, 171-186.
- Jameel S, 1999. Molecular biology and pathogenesis of hepatitis E virus. *Expert Rev Mol Med*, 1999, 1-16.
- Johne R, Plenge-Bonig A, Hess M, Ulrich RG, Reetz J and Schielke A, 2010. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. *J Gen Virol*, 91, 750-758.
- Jones MS, Lukashov VV, Ganac RD and Schnurr DP, 2007. Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. *J Clin Microbiol*, 45, 2144-2150.
- Joshi MS, Walimbe AM and Chitambar SD, 2008. Evaluation of genomic regions of hepatitis A virus for phylogenetic analysis: Suitability of the 2C region for genotyping. *J Virol Methods*, 153, 36-42.
- Jothikumar N, Cromeans TL, Robertson BH, Meng XJ and Hill VR, 2006. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods*, 131, 65-71.
- Jothikumar N, Lowther JA, Henshilwood K, Lees DN, Hill VR and Vinje J, 2005. Rapid and sensitive detection of noroviruses by using TaqMan-based one-step reverse transcription-PCR assays and application to naturally contaminated shellfish samples. *Appl Environ Microbiol*, 71, 1870-1875.
- Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, Cointault O, Esposito L, Abravanel F, Danjoux M, Durand D, Vinel JP, Izopet J and Rostaing L, 2008. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med*, 358, 811-817.
- Karsten C, Baumgarte S, Friedrich AW, von Eiff C, Becker K, Wosniok W, Ammon A, Bockemuhl J, Karch H and Huppertz HI, 2009. Incidence and risk factors for community-acquired acute gastroenteritis in north-west Germany in 2004. *Eur J Clin Microbiol Infect Dis*, 28, 935-943.
- Kasorndorkbua C, Guenette DK, Huang FF, Thomas PJ, Meng XJ and Halbur PG, 2004. Routes of transmission of swine hepatitis E virus in pigs. *J Clin Microbiol*, 42, 5047-5052.
- Khadre MA and Yousef AE, 2002. Susceptibility of human rotavirus to ozone, high pressure, and pulsed electric field. *Journal of Food Protection*
- Kiely M, Flynn A, Harrington KE, Robson PJ and Cran G, 2001. Sampling description and procedures used to conduct the North/South Ireland Food Consumption Survey. *Public Health Nutr*, 4, 1029-1035.
- Kingsley DH, Chen HQ and Hoover DG, 2004. Inactivation of selected picornaviruses by high hydrostatic pressure. *Virus Research*, 102, 221-224.

- Kingsley DH, Guan DS and Hoover DG, 2005. Pressure inactivation of hepatitis A virus in strawberry puree and sliced green onions. *Journal of Food Protection*, 68, 1748-1751.
- Kingsley DH, Hollinian DR, Calci KR, Chen HQ and Flick GJ, 2007. Inactivation of a norovirus by high-pressure processing. *Applied and Environmental Microbiology*, 73, 581-585.
- Kingsley DH, Hoover DG, Papafragkou E and Richards GP, 2002a. Inactivation of hepatitis A virus and a calicivirus by high hydrostatic pressure. *J Food Prot*, 65, 1605-1609.
- Kingsley DH, Meade GK and Richards GP, 2002b. Detection of both hepatitis A virus and Norwalk-like virus in imported clams associated with food-borne illness. *Appl Environ Microbiol*, 68, 3914-3918.
- Kingsley DH and Richards GP, 2003. Persistence of hepatitis A virus in oysters. *J Food Prot*, 66, 331-334.
- Kobayashi S, Natori K, Takeda N and Sakae K, 2004. Immunomagnetic capture rt-PCR for detection of norovirus from foods implicated in a foodborne outbreak. *Microbiol Immunol*, 48, 201-204.
- Koopmans M and Duizer E, 2004. Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90, 23-41.
- Koopmans M, Vennema H, Heersma H, van Strien E, van Duynhoven Y, Brown D, Reacher M and Lopman B, 2003. Early identification of common-source foodborne virus outbreaks in Europe. *Emerg Infect Dis*, 9, 1136-1142.
- Krems C, Bauch A, Gotez A, Heuer T, Hild A, Möseneder J and Borombach C 2006. Methoden der nationalen Verzehrstudie II. *Ernährungs umschau* 53, Heft 2, 44-50.
- Kroneman A, Verhoef L, Harris J, Vennema H, Duizer E, van Duynhoven Y, Gray J, Iturriza M, Bottiger B, Falkenhorst G, Johnsen C, von Bonsdorff CH, Maunula L, Kuusi M, Pothier P, Gallay A, Schreier E, Hohne M, Koch J, Szucs G, Reuter G, Krisztalovics K, Lynch M, McKeown P, Foley B, Coughlan S, Ruggeri FM, Di Bartolo I, Vainio K, Isakbaeva E, Poljsak-Prijatelj M, Grom AH, Mijovski JZ, Bosch A, Buesa J, Fauquier AS, Hernandez-Pezzi G, Hedlund KO and Koopmans M, 2008. Analysis of integrated virological and epidemiological reports of norovirus outbreaks collected within the Foodborne Viruses in Europe network from 1 July 2001 to 30 June 2006. *J Clin Microbiol*, 46, 2959-2965.
- Kukkula M, Maunula L, Silvennoinen E and von Bonsdorff CH, 1999. Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *J Infect Dis*, 180, 1771-1776.
- Kumar A, Beniwal M, Kar P, Sharma JB and Murthy NS, 2004. Hepatitis E in pregnancy. *Int J Gynaecol Obstet*, 85, 240-244.
- Kuo DH, Simmons FJ, Blair S, Hart E, Rose JB and Xagorarakis I, 2010. Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. *Water Research*, 44, 1520-1530.
- Kuo HW, Schmid D, Jelovcan S, Pichler AM, Magnet E, Reichart S and Allerberger F, 2009. A foodborne outbreak due to norovirus in Austria, 2007. *J Food Prot*, 72, 193-196.
- Kurdtziel AS, Wilkinson N, Langton S and Cook N, 2001. Survival of poliovirus on soft fruit and salad vegetables. *J Food Prot*, 64, 706-709.
- Kuusi M, Nuorti JP, Maunula L, Minh Tran NN, Ratia M, Karlsson J and von Bonsdorff CH, 2002. A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. *Epidemiol Infect*, 129, 133-138.
- Lamhoujeb S, Fliss I, Ngazoa SE and Jean J, 2009. Molecular study of the persistence of infectious human norovirus on food-contact surfaces. *Food and Environmental Virology*, 1, 51-56.

- Lau CS, Wong DA, Tong LK, Lo JY, Ma AM, Cheng PK and Lim WW, 2004a. High rate and changing molecular epidemiology pattern of norovirus infections in sporadic cases and outbreaks of gastroenteritis in Hong Kong. *J Med Virol*, 73, 113-117.
- Lau SK, Woo PC, Wong BH, Tsoi HW, Woo GK, Poon RW, Chan KH, Wei WI, Peiris JS and Yuen KY, 2004b. Detection of severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein in sars patients by enzyme-linked immunosorbent assay. *J Clin Microbiol*, 42, 2884-2889.
- Le Guyader F and Atmar RL, 2008. Binding and inactivation of viruses on and in food, with a focus on the role of the matrix. In: *Food-borne viruses: progress and challenges*. Eds: M Koopmans, A Bosch, D Cleaver. ASM Press, Washington DC, 189-208.
- Le Guyader F, Haugarreau L, Miossec L, Dubois E and Pommepuy M, 2000. Three-year study to assess human enteric viruses in shellfish. *Appl Environ Microbiol*, 66, 3241-3248.
- Le Guyader FS, Bon F, DeMedici D, Parnaudeau S, Bertone A, Crudeli S, Doyle A, Zidane M, Suffredini E, Kohli E, Maddalo F, Monini M, Gallay A, Pommepuy M, Pothier P and Ruggeri FM, 2006a. Detection of multiple noroviruses associated with an international gastroenteritis outbreak linked to oyster consumption. *J Clin Microbiol*, 44, 3878-3882.
- Le Guyader FS, Krol J, Ambert-Balay K, Ruvoen-Clouet N, Desaubliaux B, Parnaudeau S, Le Saux JC, Ponge A, Pothier P, Atmar RL and Le Pendu J, 2010. Comprehensive analysis of a norovirus-associated gastroenteritis outbreak, from the environment to the consumer. *J Clin Microbiol*, 48, 915-920.
- Le Guyader FS, Le Saux JC, Ambert-Balay K, Krol J, Serais O, Parnaudeau S, Giraudon H, Delmas G, Pommepuy M, Pothier P and Atmar RL, 2008. Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *J Clin Microbiol*, 46, 4011-4017.
- Le Guyader FS, Loisy F, Atmar RL, Hutson AM, Estes MK, Ruvoen-Clouet N, Pommepuy M and Le Pendu J, 2006b. Norwalk virus-specific binding to oyster digestive tissues. *Emerging Infectious Diseases*, 12, 931-936.
- Le Guyader FS, Neill FH, Dubois E, Bon F, Loisy F, Kohli E, Pommepuy M and Atmar RL, 2003. A semiquantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak. *Int J Food Microbiol*, 87, 107-112.
- Le Guyader FS, Parnaudeau S, Schaeffer J, Bosch A, Loisy F, Pommepuy M and Atmar RL, 2009. Detection and quantification of noroviruses in shellfish. *Appl Environ Microbiol*, 75, 618-624.
- Leclercq C, Arcella D, Piccinelli R, Sette S, Le Donne C and Turrini A, 2008. The Italian national food consumption survey INRAN-SCAI 2005-06: main results in terms of food consumption. *Public Health Nutrition*, 1-29,
- Lederer I, Schmid D, Pichler AM, Dapra R, Kraler P, Blassnig P, Luckner-Hornischer A, Berghold C and Allerberger F, 2005. Outbreak of norovirus infections associated with consuming food from a catering company, Austria. *Eurosurveillance*, 10,
- Lees D, 2000. Viruses and bivalve shellfish. *Int J Food Microbiol*, 59, 81-116.
- Lees D and CW T, 2010. International Standardisation of a Method for Detection of Human Pathogenic Viruses in Molluscan Shellfish. *Food and Environ*, 2, 146-155.
- Lewis HC, Wichmann O and Duizer E, 2010. Transmission routes and risk factors for autochthonous hepatitis E virus infection in Europe: a systematic review. *Epidemiol Infect*, 138, 145-166.
- Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Kurata Y, Ishida M, Sakamoto S, Takeda N and Miyamura T, 2005. Hepatitis E virus transmission from wild boar meat. *Emerg Infect Dis*, 11, 1958-1960.
- Li TC, Miyamura T and Takeda N, 2007. Detection of hepatitis E virus RNA from the bivalve Yamato-Shijimi (*Corbicula japonica*) in Japan. *Am J Trop Med Hyg*, 76, 170-172.

- Lindesmith L, Moe CL, Marionneau S, Ruvoen N, Jiang X, Lindbland L, Stewart P, le Pendu J and Baric R, 2003. Human susceptibility and resistance to Norwalk virus infection. *Nature Medicine*, 9, 548–553.
- Lodder-Verschuur F, de Roda Husman AM, van den Berg HH, Stein A, van Pelt-Heerschap HM and van der Poel WH, 2005. Year-round screening of noncommercial and commercial oysters for the presence of human pathogenic viruses. *J Food Prot*, 68, 1853-1859.
- Lodder WJ and de Roda Husman AM, 2005. Presence of noroviruses and other enteric viruses in sewage and surface waters in The Netherlands. *Appl Environ Microbiol*, 71, 1453-1461.
- Loisy F, Atmar RL, Le Saux JC, Cohen JI, Caprais MP, Pommepuy M and Le Guyader FS, 2005. Use of rotavirus virus-like particles as surrogates to evaluate virus persistence in shellfish. *Applied and Environmental Microbiology*, 71, 6049-6053.
- Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negrodo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M, Gallimore CI, Bottiger B, Hedlund KO, Torvén M, von Bonsdorff CH, Maunula L, Poljsak-Prijatelj M, Zimsek J, Reuter G, Szűcs G, Melegh B, Svennson L, van Duynhoven Y and Koopmans M, 2004. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. *Lancet*, 363, 682-688.
- Lopman BA, Reacher MH, Van Duynhoven Y, Hanon FX, Brown D and Koopmans M, 2003. Viral gastroenteritis outbreaks in Europe, 1995-2000. *Emerg Infect Dis*, 9, 90-96.
- Lowther JA, Avant JM, Gizynski K, Rangdale RE and Lees DN, 2010. Comparison between quantitative real-time reverse transcription PCR results for norovirus in oysters and self-reported gastroenteric illness in restaurant customers. *J Food Prot*, 73, 305-311.
- Lowther JA, Henshilwood K and Lees DN, 2008. Determination of norovirus contamination in oysters from two commercial harvesting areas over an extended period, using semiquantitative real-time reverse transcription PCR. *J Food Prot*, 71, 1427-1433.
- Lu L, Ching KZ, de Paula VS, Nakano T, Siegl G, Weitz M and Robertson BH, 2004. Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). *J Gen Virol*, 85, 2943-2952.
- Lu L, Li C and Hagedorn CH, 2006. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Reviews Medical Virology*, 16, 5-36.
- Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, Gurley E, Khan R, Ahmed BN, Rahman S, Nahar N, Kenah E, Comer JA and Ksiazek TG, 2006. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis*, 12, 1888-1894.
- Lyhne N, Christensen T, Velsing G, Fagt S, Bilstoft-Jensen A, Hartkopp H, Hinsch HJ, Matthiesen J, Moller A, Saxholt E and Trolle E, 2005. Danskernes kostvaner 2000-2002. Hovedresultater. *Danmarks Fodevareforskning*, 1-168.
- Maalouf H, Pommepuy M and Le Guyader FS, 2010a. Environmental conditions leading to shellfish contamination and related outbreaks. *Food Environ Virol*, 2, 136-145.
- Maalouf H, Zakhour M, Le Pendu J, Le Saux JC, Atmar RL and Le Guyader FS, 2010b. Distribution in tissue and seasonal variation of norovirus genogroup I and II ligands in oysters. *Appl Environ Microbiol*, 76, 5621-5630.
- Macaluso A, Petrinca A, Lanni L, Saccares S, Amiti S, Gabrieli R and Divizia M, 2006. Identification and sequence analysis of hepatitis A virus detected in market and environmental bivalve molluscs. *J Food Prot*, 69, 449-452.
- Mackiewicz V, Cammas A, Desbois D, Marchadier E, Pierredon S, Beaulieux F, Dussaix E, Vagner S and Roque-Afonso AM, 2010. Nucleotide variability and translation efficiency of the 5' untranslated region of hepatitis A virus: update from clinical isolates associated with mild and severe hepatitis. *J Virol*, 84, 10139-10147.

- Mahony JO, Donoghue MO, Morgan JG and Hill C, 2000. Rotavirus survival and stability in foods as determined by an optimised plaque assay procedure. *International Journal of Food Microbiology*, 61, 177-185.
- Mallett JC, Beghian LE, Metcalf TG and Kaylor JD, 1991. Potential of irradiation technology for improved shellfish sanitation. *Journal of Food Safety*, 11, 231-245.
- Manso CF, Polo D, Vilarino ML and Romalde JL, 2010. Genotyping of hepatitis A virus detected in bivalve shellfish in Galicia (NW Spain). *Water Sci Technol*, 61, 15-24.
- Marshall JA and Bruggink LD, 2006. Laboratory diagnosis of norovirus. *Clin Lab*, 52, 571-581.
- Marshall JA, Hellard ME, Sinclair MI, Fairley CK, Cox BJ, Catton MG, Kelly H and Wright PJ, 2003. Incidence and characteristics of endemic Norwalk-like virus-associated gastroenteritis. *J Med Virol*, 69, 568-578.
- Martinez N, Espul C, Cuello H, Zhong W, Jiang X, Matson DO and Berke T, 2002. Sequence diversity of human caliciviruses recovered from children with diarrhea in Mendoza, Argentina, 1995-1998. *J Med Virol*, 67, 289-298.
- Masuda J, Yano K, Tamada Y, Takii Y, Ito M, Omagari K and Kohno S, 2005. Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. *Hepatol Res*, 31, 178-183.
- Matsuda H, Okada K, Takahashi K and Mishiro S, 2003. Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *J Infect Dis*, 188, 944.
- Mattison K and Bidawid S, 2009. Analytical methods for food and environmental viruses. *Food Environ Virol*, 1, 107-122.
- Mattison K, Brassard J, Gagne MJ, Ward P, Houde A, Lessard L, Simard C, Shukla A, Pagotto F, Jones TH and Trottier YL, 2009. The feline calicivirus as a sample process control for the detection of food and waterborne RNA viruses. *International Journal of Food Microbiology*, 132, 73-77.
- Mattison K, Karthikeyan K, Abebe M, Malik N, Sattar SA, Farber JM and Bidawid S, 2007. Survival of calicivirus in foods and on surfaces: experiments with feline calicivirus as a surrogate for norovirus. *J Food Prot*, 70, 500-503.
- Maunula L, Roivainen M, Keranen M, Makela S, Soderberg K, Summa M, von Bonsdorff CH, Lappalainen M, Korhonen T, Kuusi M and Niskanen T, 2009. Detection of human norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks. *Euro Surveill*, 14,
- Mbithi JN, Springthorpe VS and Sattar SA, 1991. Effect of relative humidity and air temperature on survival of hepatitis A virus on environmental surfaces. *Applied and Environmental Microbiology*, 57, 1349-1399.
- McCaustland KA, Bond WW, Bradley DW, Ebert JW and Maynard JE, 1982. Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol*, 16, 957-958.
- Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM and Tauxe RV, 1999. Food-related illness and death in the United States. *Emerg Infect Dis*, 5, 607-625.
- Metcalf TG, Moulton E and Eckerson D, 1980. Improved method and test strategy for recovery of enteric viruses from shellfish. *Appl Environ Microbiol*, 39, 141-152.
- Millard J, Appleton H and Parry JV, 1987. Studies on heat inactivation of hepatitis A virus with special reference to shellfish. 1. Procedures for infection and recovery of virus from laboratory maintained cockles. *Epidemiology and Infection*, 98, 397-414.
- Mokhtari A and Jaykus LA, 2009. Quantitative exposure model for the transmission of norovirus in retail food preparation. *Int J Food Microbiol*, 133, 38-47.
- Morton V, Jean J, Farber J and Mattison K, 2009. Detection of noroviruses in ready-to-eat foods by using carbohydrate-coated magnetic beads. *Appl Environ Microbiol*, 75, 4641-4643.

- Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J and Glass RI, 2000. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis*, 181 Suppl 2, S284-287.
- Muniain-Mujika I, Calvo M, Lucena F and Girones R, 2003. Comparative analysis of viral pathogens and potential indicators in shellfish. *Int J Food Microbiol*, 83, 75-85.
- Munoz I, Tomas N, Mas J, Garcia-Reyes JF, Molina-Diaz A and Fernandez-Alba AR, 2010. Potential chemical and microbiological risks on human health from urban wastewater reuse in agriculture. Case study of wastewater effluents in Spain. *J Environ Sci Health B*, 45, 300-309.
- Myrmel M, Berg EM, Rimstad E and Grinde B, 2004. Detection of enteric viruses in shellfish from the Norwegian coast. *Appl Environ Microbiol*, 70, 2678-2684.
- Nainan OV, Xia G, Vaughan G and Margolis HS, 2006. Diagnosis of hepatitis a virus infection: a molecular approach. *Clin Microbiol Rev*, 19, 63-79.
- Nicand E, Bigaillon C and Tessé S, 2009. Hepatite E en France: données de surveillance des cas humains, 2006-2008. *Bulletin Epidemiologie Hebdomadaire*, 31-32, 337-343.
- Nishida T, Kimura H, Saitoh M, Shinohara M, Kato M, Fukuda S, Munemura T, Mikami T, Kawamoto A, Akiyama M, Kato Y, Nishi K, Kozawa K and Nishio O, 2003. Detection, quantitation, and phylogenetic analysis of noroviruses in Japanese oysters. *Appl Environ Microbiol*, 69, 5782-5786.
- Nishida T, Nishio O, Kato M, Chuma T, Kato H, Iwata H and Kimura H, 2007. Genotyping and quantitation of noroviruses in oysters from two distinct sea areas in Japan. *Microbiol Immunol*, 51, 177-184.
- Noble RC, Kane MA, Reeves SA and Roeckel I, 1984. Posttransfusion hepatitis A in a neonatal intensive care unit. *JAMA*, 252, 2711-2715.
- Noda M, Fukuda S and Nishio O, 2008. Statistical analysis of attack rate in norovirus foodborne outbreaks. *International Journal of Food Microbiology*, 122, 216-220.
- Ocké MC, Hulshof KFAM and van Rossum CTM, 2005. The dutch national food consumption survey 2003. Methodological issues. *Arch Public Health*, 63, 227-241.
- Ozawa K, Oka T, Takeda N and Hansman GS, 2007. Norovirus infections in symptomatic and asymptomatic food handlers in Japan. *Journal of Clinical Microbiology*, 45, 3996-4005.
- Pajan-Lehpaner G and Petrak O, 2009. A one year retrospective study of gastroenteritis outbreaks in Croatia: incidences and etiology. *Coll Antropol*, 33, 1139-1144.
- Parks CG, Moe CL, Rhodes D, Lima A, Barrett L, Tseng F, Baric R, Talal A and Guerrant R, 1999. Genomic diversity of "Norwalk like viruses" (NLVs): pediatric infections in a Brazilian shantytown. *J Med Virol*, 58, 426-434.
- Parrino TA, Schreiber DS, Trier JS, Kapikian AZ and Blacklow NR, 1977. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med*, 297, 86-89.
- Parshionikar SU, Willian-True S, Fout GS, Robbins DE, Seys SA, Cassady JD and Harris R, 2003. Waterborne outbreak of gastroenteritis associated with a norovirus. *Appl Environ Microbiol*, 69, 5263-5268.
- Paturi M, Tapanainen H and Reinivuo H (National Public Health Institute), 2008. The National FINDIET 2007 Survey. Available at: (http://www.ktl.fi/attachments/suomi/julkaisut/julkaisusarja_b/2008/2008b23.pdf). No. B23.
- Pavio N, Meng XJ and Renou C, 2010. Zoonotic hepatitis E: animal reservoirs and emerging risks. *Vet Res*, 41, 46.
- Payne KJ, Hall M, Lutzke M, Armstrong C and King J, 2006. Multisite outbreak of Norovirus associated with a franchise restaurant, Kent County, Michigan. *Morbidity and Mortality Weekly Report*, 55, 395-397.

- Petterson SR and Ashbolt NJ, 2001. Viral risks associated with wastewater reuse: modeling virus persistence on wastewater irrigated salad crops. *Water Sci Technol*, 43, 23-26.
- Petterson SR, Ashbolt NJ and Sharma A, 2001. Microbial risks from wastewater irrigation of salad crops: a screening-level risk assessment. *Water Environ Res*, 73, 667-672.
- Phan TG, Okame M, Nguyen TA, Maneekarn N, Nishio O, Okitsu S and Ushijima H, 2004. Human astrovirus, norovirus (GI, GII), and sapovirus infections in Pakistani children with diarrhea. *J Med Virol*, 73, 256-261.
- Phillips G, Lopman B, Tam CC, Iturriza-Gomara M, Brown D and Gray J, 2009. Diagnosing norovirus-associated infectious intestinal disease using viral load. *BMC Infect Dis*, 9, 63.
- Pinto RM, Alegre D, Dominguez A, El-Senousy WM, Sanchez G, Villena C, Costafreda MI, Aragonés L and Bosch A, 2007. Hepatitis A virus in urban sewage from two Mediterranean countries. *Epidemiol Infect*, 135, 270-273.
- Pinto RM and Bosch A, 2008. Rethinking virus detection in food. In: *Food-borne viruses progress and challenges*. Eds: M Koopmans, DO Cliver, A Bosch. ASM Press, Washington D.C., 171-188.
- Pinto RM, Costafreda MI and Bosch A, 2009. Risk assessment in shellfish-borne outbreaks of hepatitis A. *Appl Environ Microbiol*, 75, 7350-7355.
- Pomerleau J, McKee M, Robertson A, Vaask S, Pudule I, Grinberga D, Kadziauskiene K, Abaravicius A and Bartkeviciute R 1999. Nutrition and lifestyle in the Baltic Republics. European centre on health of societies in transition and world health organization regional office for Europe, 1-61.
- Ponka A, Maunula L, von Bonsdorff CH and Lyytikäinen O, 1999. An outbreak of calicivirus associated with consumption of frozen raspberries. *Epidemiol Infect*, 123, 469-474.
- Preiss JC, Plentz A, Engelmann E, Schneider T, Jilg W, Zeitz M and Duchmann R, 2006. Autochthonous hepatitis E virus infection in Germany with sequence similarities to other European isolates. *Infection*, 34, 173-175.
- Previsani N, Lavanchy D and Siegl G, 2004. Hepatitis A. In: *Viral Hepatitis Molecular Biology, Diagnosis, Epidemiology and Control*. IK Mushahwar. Elsevier, 1-30.
- Purcell RH and Emerson SU, 2001. Hepatitis E Virus. In: *Fields Virology*. Eds: DM Knipe, PM Howe. Raven Press, New York, 3051-3061.
- Ramsay CN and Upton PA, 1989. Hepatitis A and frozen raspberries. *Lancet*, 1, 43-44.
- Regli S, B. RJ, Haas CN and Gerba CP, 1991. Modeling the risk from Giardia and viruses in drinking water. *J Am Wat Works Assoc*, 83, 76-84.
- Reid TM and Robinson HG, 1987. Frozen raspberries and hepatitis A. *Epidemiol Infect*, 98, 109-112.
- Requejo Marcos A, Ortega Anta R, Lopez Sobaler AM, Navia Lomban B, Andres Carvajales P, Jodral Villarejo M, Quintas Herrero E, Redondo Sobrado R, Menendez Bueno L, Perea Sanchez JM, Aparicio Vizuete A, Lozano Estevan C, Bermejo Alonso L, Mena Valerde C, Faci Veja M, Lolo Velarde JM, Rodriguez N, Cocho Rodriguez M, Diez de la Cortina C and Ivarez Sanematerio C (Consejo de seguridad nuclear), 2002. Estudio sobre dietas y hábitos alimentarios en la población Española. 1-303.
- Reuter G, Farkas T, Berke T, Jiang X, Matson DO and Szűcs G, 2002. Molecular epidemiology of human calicivirus gastroenteritis outbreaks in Hungary, 1998 to 2000. *J Med Virol*, 68, 390-398.
- Richards GP, McLeod C and Le Guyader FS, 2010. Processing strategies to inactivate enteric viruses in shellfish: limitations of surrogate viruses and molecular methods. *Food Environ Virol*, 2, 183-193.
- Rizzo C, Di Bartolo I, Santantonio M, Coscia MF, Monno R, De Vito D, Ruggeri FM and Rizzo G, 2007. Epidemiological and virological investigation of a norovirus outbreak in a resort in Puglia, Italy. *BMC Infectious Diseases*, 7, 135.

- Roberts P and Hope A, 2003. Virus inactivation by high intensity broad spectrum pulsed light. *Journal of Virological Methods*, 110, 61-65.
- Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, Widell A, Margolis HS, Isomura S, Ito K and et al., 1992. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J Gen Virol*, 73, 1365-1377.
- Robertson BH, Khanna B, Nainan OV and Margolis HS, 1991. Epidemiologic patterns of wild-type hepatitis A virus determined by genetic variation. *J Infect Dis*, 163, 286-292.
- Rodler I, Biró L, Greiner E, Zajkás G, Szórád I, Varga A, Domonkos A, Agoston H, Balazs A, Mozsary E, Vitrai J, Hermann D, Boros J, Nemeth R and Zsuzsanna K, 2005. Taplalkozasi vizsgalat Magyarorszagon, 2003-2004. *Evolyam*, 34, 1781-1789.
- Roos B, 1956. [Hepatitis epidemic transmitted by oysters.]. *Sven Lakartidn*, 53, 989-1003.
- Roseblum LS, Mirkin IR, Allen DT, Safford S and Hadler SC, 1990. A multistate outbreak of hepatitis A traced to commercially distributed lettuce. *American Journal of Public Health*, 80, 1075-1080.
- Ruprich J, Dofkova M, Rehurkova I, Slamenikova E and Resova D 2006. Individual food consumption-the national study SISP04. CHFCH NIPH in Prague, available at URL: <http://www.chpr.szu.cz/spotrebapotravin.htm>.
- Rzezutka A and Cook N, 2004. Survival of human enteric viruses in the environment and food. *FEMS Microbiol Rev*, 28, 441-453.
- Sakon N, Yamazaki K, Yoda T, Kanki M, Otake T and Tsukamoto T, 2005. A norovirus outbreak of gastroenteritis linked to packed lunches. *Japanese Journal of Infectious Diseases*, 58, 253.
- Sanchez G, Bosch A, Gomez-Mariano G, Domingo E and Pinto RM, 2003a. Evidence for quasispecies distributions in the human hepatitis A virus genome. *Virology*, 315, 34-42.
- Sanchez G, Bosch A and Pinto RM, 2003b. Genome variability and capsid structural constraints of hepatitis a virus. *J Virol*, 77, 452-459.
- Sanchez G, Pinto RM, Vanaclocha H and Bosch A, 2002. Molecular characterization of hepatitis a virus isolates from a transcontinental shellfish-borne outbreak. *J Clin Microbiol*, 40, 4148-4155.
- Šantare D, Ozoliņš G and Joffe R 2008. Latvijas iedzīvotāju pārtikas patēriņa pētījums: mērķi, norise, metodes. LLU Raksti, Jelgava, iesniegta publicēšanai.
- Scherer K, Made D, Ellerbroek L, Schulenburg J, Johne R and Klein G, 2009. Application of a swab sampling method for the detection of norovirus and rotavirus to artificially contaminated food and environmental surfaces. *Food and Environmental Virology*, 1, 42-49.
- Schielke A, Sachs K, Lierz M, Appel B, Jansen A and Johne R, 2009. Detection of hepatitis E virus in wild boars of rural and urban regions in Germany and whole genome characterization of an endemic strain. *Virol J*, 6, 58.
- Schlauder GG, Desai SM, Zanetti AR, Tassopoulos NC and Mushahwar IK, 1999. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. *J Med Virol*, 57, 243-251.
- Schmid D, Stuger HP, Lederer I, Pichler AM, Kainz-Arnfelder G, Schreier E and Allerberger F, 2007. A foodborne norovirus outbreak due to manually prepared salad, Austria 2006. *Infection*, 35, 232-239.
- Scholz E, Heinricy U and Flehmig B, 1989. Acid stability of hepatitis A virus. *Journal of General Virology*, 70, 2481-2485.
- Schultheiss T, Emerson SU, Purcell RH and Gauss-Muller V, 1995. Polyprotein processing in echovirus 22: a first assessment. *Biochem Biophys Res Commun*, 217, 1120-1127.

- Schultheiss T, Kusov YY and Gauss-Muller V, 1994. Proteinase 3C of hepatitis A virus (HAV) cleaves the HAV polyprotein P2-P3 at all sites including VP1/2A and 2A/2B. *Virology*, 198, 275-281.
- Schwab KJ, Neill FH, Estes MK, Metcalf TG and Atmar RL, 1998. Distribution of norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR. *Journal of Food Protection*, 61, 1674-1680.
- Schwab KJ, Neill FH, Fankhauser RL, Daniels NA, Monroe SS, Bergmire-Sweet DA, Estes MK and Atmar RL, 2000. Development of methods to detect "Norwalk-like viruses" (NLVs) and hepatitis A virus in delicatessen foods: application to a food-borne NLV outbreak. *Appl Environ Microbiol*, 66, 213-218.
- Sekula W, Nelson M, Figurska K, Oltarzewski M, Weisell R and Szponar L, 2004. Comparison between household budget survey and 24 hour recall data in a nationally representative sample of Polish households. *Public Health Nutrition*, 8, 430-439.
- Sharma M, Shearer AEH, Hoover DG, Liu MN, Solomon MB and Kniel KE, 2008. Comparison of hydrostatic and hydrodynamic pressure to inactivate foodborne viruses. *Innovative Food Science & Emerging Technologies*, 9, 418-422.
- Sheretz RJ, Russell BA and Reunman PD, 2005. Transmission of hepatitis A by transfusion of blood products. *Archives of Internal Medicine*, 1441, 1579-1580.
- Shieh YC, Khudyakov YE, Xia G, Ganova-Raeva LM, Khambaty FM, Woods JW, Veazey JE, Motes ML, Glatzer MB, Bialek SR and Fiore AE, 2007. Molecular confirmation of oysters as the vector for hepatitis A in a 2005 multistate outbreak. *J Food Prot*, 70, 145-150.
- Shuval H, Lampert Y and Fattal B, 1997. Development of a risk assessment model for evaluating wastewater reuse standards for agriculture. *Wat Sci Tech*, 35, 15-20.
- Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G and Koopmans M, 2009. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. *J Infect Dis*, 200, 802-812.
- Simmons G, Garbutt C, Hewitt J and Greening G, 2007. A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters. *New Zealand Medical Journal*, 120, 2773.
- Sincero TC, Levin DB, Simoes CM and Barardi CR, 2006. Detection of hepatitis A virus (HAV) in oysters (*Crassostrea gigas*). *Water Res*, 40, 895-902.
- Sjogren MH, Tanno H, Fay O, Sileoni S, Cohen BD, Burke DS and Feighny RJ, 1987. Hepatitis A virus in stool during clinical relapse. *Ann Intern Med*, 106, 221-226.
- Slomka MJ and Appleton H, 1998. Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. *Epidemiology and Infection*, 121, 401-407.
- Sobsey MD, Shields PA, Hauchman FH, Hazard RL and Caton LW, 1989. Survival and transport of hepatitis A virus in soils, groundwater and wastewater. *Water Sci Technol*, 18, 97-106.
- Sobsey MD, Shields PA, Hauchman FS, Davies AL, Rullman VA and Bosch A, 1988. Survival and persistence of hepatitis A virus in environmental samples. In: *Viral Hepatitis and Liver Disease*. Eds: AJ Zuckerman. Alan R. Liss, Inc, New York, 121-124.
- Son NT and Fleet GH, 1980. Behavior of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying, and storage. *Applied and Environmental Microbiology*, 40, 994-1002.
- Springthorpe VS, Loh CL, Robertson WJ and Sattar SA, 1993. In situ survival of indicator bacteria, MS-2 phage and human pathogenic viruses in river water. *Water Sci Technol*, 27, 413-420.

- Stals A, Baert L, Jasson V, Van Coillie E and Uyttendaele M, 2011. Screening of fruit products for norovirus and the difficulty of interpreting positive PCR results. *Journal of Food Protection*, 74:425-431.
- Stene-Johansen K, Jenum PA, Hoel T, Blystad H, Sunde H and Skaug K, 2002. An outbreak of hepatitis A among homosexuals linked to a family outbreak. *Epidemiol Infect*, 129, 113-117.
- Stene-Johansen K, Tjon G, Schreier E, Bremer V, Bruisten S, Ngui SL, King M, Pinto RM, Aragones L, Mazick A, Corbet S, Sundqvist L, Blystad H, Norder H and Skaug K, 2007. Molecular epidemiological studies show that hepatitis A virus is endemic among active homosexual men in Europe. *J Med Virol*, 79, 356-365.
- Stine SW, Song I, Choi CY and Gerba CP, 2005a. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. *J Food Prot*, 68, 913-918.
- Stine SW, Song I, Choi CY and Gerba CP, 2005b. Effect of relative humidity on preharvest survival of bacterial and viral pathogens on the surface of cantaloupe, lettuce, and bell peppers. *J Food Prot*, 68, 1352-1358.
- Strazynski M, Kramer J and Becker B, 2002. Thermal inactivation of poliovirus type 1 in water, milk and yoghurt. *International Journal of Food Microbiology*, 74, 73-78.
- Suffredini E, Corrain C, Arcangeli G, Fasolato L, Manfrin A, Rossetti E, Biazzini E, Mioni R, Pavoni E, Losio MN, Sanavio G and Croci L, 2008. Occurrence of enteric viruses in shellfish and relation to climatic-environmental factors. *Lett Appl Microbiol*, 47, 467-474.
- Sullivan R, Scarpino PV, Fassolit AC, Larkin EP and Peeler JT, 1973. Gamma radiation inactivation of coxsackie virus B-2. *Applied Microbiology*, 26, 14-17.
- Sunen E, Casas N, Moreno B and Zigorraga C, 2004. Comparison of two methods for the detection of hepatitis A virus in clam samples (*Tapes* spp.) by reverse transcription-nested PCR. *Int J Food Microbiol*, 91, 147-154.
- Svraka S, Duizer E, Vennema H, de Bruin E, van der Veer B, Dorresteyn B and Koopmans M, 2007. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. *J Clin Microbiol*, 45, 1389-1394.
- Swan G, 2004. Findings from the latest National diet and Nutrition Survey. *Proceedings of the Nutrition Society*, 63, 505-512.
- Takahashi M, Tanaka T, Azuma M, Kusano E, Aikawa T, Shibayama T, Yazaki Y, Mizuo H, Inoue J and Okamoto H, 2007. Prolonged fecal shedding of hepatitis E virus (HEV) during sporadic acute hepatitis E: evaluation of infectivity of HEV in fecal specimens in a cell culture system. *J Clin Microbiol*, 45, 3671-3679.
- Tallo T, Norder H, Tefanova V, Ott K, Ustina V, Prukk T, Solomonova O, Schmidt J, Zilmer K, Priimagi L, Krispin T and Magnius LO, 2003. Sequential changes in hepatitis A virus genotype distribution in Estonia during 1994 to 2001. *J Med Virol*, 70, 187-193.
- Tanaka J, 2000. Hepatitis A shifting epidemiology in Latin America. *Vaccine*, 18 Suppl 1, S57-60.
- Tanaka T, Takahashi M, Kusano E and Okamoto H, 2007. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. *J Gen Virol*, 88, 903-911.
- Tei S, Kitajima N, Takahashi K and Mishiro S, 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*, 362, 371-373.
- Teo CG, 2009. Much meat, much malady: changing perceptions of the epidemiology of hepatitis E. *Clin Microbiol Infect*, 16, 24-32.
- Teppakdee A, Tangwitoon A, Khemasuwan D, Tangdhanakanond K, Suramaethakul N, Sriratanaban J and Poovorawan Y, 2002. Cost-benefit analysis of hepatitis a vaccination in Thailand. *Southeast Asian J Trop Med Public Health*, 33, 118-127.

- ter Waarbeek HL, Dukers-Muijers NH, Vennema H and Hoebe CJ, 2010. Waterborne gastroenteritis outbreak at a scouting camp caused by two norovirus genogroups: GI and GII. *Journal of Clinical Virology*, 47, 268-272.
- Terio V, Martella V, Moschidou P, Di Pinto P, Tantillo G and Buonavoglia C, 2010. Norovirus in retail shellfish. *Food Microbiol*, 27, 29-32.
- Terpstra FG, van den Blink AE, Bos LM, Boots AG, Brinkhuis FH, Gijsen E, van Remmerden Y, Schuitemaker H and van 't Wout AB, 2007. Resistance of surface-dried virus to common disinfection procedures. *J Hosp Infect*, 66, 332-338.
- Teunis PF, Moe CL, Liu P, Miller SE, Lindesmith L, Baric RS, Le Pendu J and Calderon RL, 2008. Norwalk virus: how infectious is it? *Journal of Medical Virology*, 80, 1468-1476.
- Tian P, Engelbrekton AL, Jiang X, Zhong WM and Mandrelli RE, 2007. Norovirus recognizes histo-blood group antigens on gastrointestinal cells of clams, mussels, and oysters: a possible mechanism of bioaccumulation. *Journal of Food Protection*, 70, 2140-2147.
- Todd EC, Greig JD, Bartleson CA and Michaels BS, 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 2. Description of outbreaks by size, severity, and settings. *J Food Prot*, 70, 1975-1993.
- Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, Owen RJ, Brazier JS, Cumberland P, King V and Cook PE, 1999. A study of infectious intestinal disease in England: microbiological findings in cases and controls. *Commun Dis Public Health*, 2, 108-113.
- Tortajada C, de Olalla PG, Pinto RM, Bosch A and Cayla J, 2009. Outbreak of hepatitis A among men who have sex with men in Barcelona, Spain, September 2008-March 2009. *Euro Surveill*, 14,
- Tsarev SA, Tsareva TS, Emerson SU, Yarbough PO, Legters LJ, Moskal T and Purcell RH, 1994. Infectivity titration of a prototype strain of hepatitis E virus in cynomolgus monkeys. *J Med Virol*, 43, 135-142.
- Tumpey TM, Suarez DL, Perkins LE, Senne DA, Lee J, Lee YJ, Mo IP, Sung HW and Swayne DE, 2003. Evaluation of a high-pathogenicity H5N1 avian influenza A virus isolated from duck meat. *Avian Dis*, 47, 951-955.
- Turcios RM, Widdowson MA, Sulka AC, Mead PS and Glass RI, 2006. Reevaluation of epidemiological criteria for identifying outbreaks of acute gastroenteritis due to norovirus: United States, 1998-2000. *Clin Infect Dis*, 42, 964-969.
- Ueki Y, Sano D, Watanabe T, Akiyama K and Omura T, 2005. Norovirus pathway in water environment estimated by genetic analysis of strains from patients of gastroenteritis, sewage, treated wastewater, river water and oysters. *Water Res*, 39, 4271-4280.
- Ueki Y, Shoji M, Suto A, Tanabe T, Okimura Y, Kikuchi Y, Saito N, Sano D and Omura T, 2007. Persistence of caliciviruses in artificially contaminated oysters during depuration. *Applied and Environmental Microbiology*, 73, 5698-5701.
- Umesha KR, Bhavani NC, Venugopal MN, Karunasagar I and Krohne G, 2008. Prevalence of human pathogenic enteric viruses in bivalve molluscan shellfish and cultured shrimp in south west coast of India. *Int J Food Microbiol*, 122, 279-286.
- Van Damme P and Van Herck K, 2005. Effect of hepatitis A vaccination programs. *JAMA*, 294, 246-248.
- van den Berg H, Lodder W, van der Poel W, Vennema H and de Roda Husman AM, 2005. Genetic diversity of noroviruses in raw and treated sewage water. *Res Microbiol*, 156, 532-540.
- Verhoef L, Kouyos RD, Vennema H, Kroneman A, Siebenga J, Van Pelt W and Koopmans M, 2011. An integrated approach to identifying international foodborne norovirus outbreaks. *Emerging Infectious Diseases*, 17:412-418.

- Verhoef LP, Kroneman A, van Duynhoven Y, Boshuizen H, van Pelt W and Koopmans M, 2009. Selection tool for foodborne norovirus outbreaks. *Emerg Infect Dis*, 15, 31-38.
- Vilarino ML, Le Guyader FS, Polo D, Schaeffer J, Krol J and Romalde JL, 2009. Assessment of human enteric viruses in cultured and wild bivalve molluscs. *Int Microbiol*, 12, 145-151.
- Vinje J, Green J, Lewis DC, Gallimore CI, Brown DW and Koopmans MP, 2000. Genetic polymorphism across regions of the three open reading frames of "Norwalk-like viruses". *Arch Virol*, 145, 223-241.
- Vinje J, Hamidjaja RA and Sobsey MD, 2004. Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. *J Virol Methods*, 116, 109-117.
- Visser H, Verhoef L, Schop W and Götz HM, 2010. Outbreak investigation in two groups of coach passengers with gastroenteritis returning from Germany to the Netherlands in February 2009. *Euro Surveill*, 15,
- Viswanathan R, 1957. *Epidemiology*. *Indian J Med Res*, 45, 1-29.
- Wasley A, Samandari T and Bell BP, 2005. Incidence of hepatitis A in the United States in the era of vaccination. *JAMA*, 294, 194-201.
- Weitz M, Baroudy BM, Maloy WL, Ticehurst JR and Purcell RH, 1986. Detection of a genome-linked protein (VPg) of hepatitis A virus and its comparison with other picornaviral VPgs. *J Virol*, 60, 124-130.
- Westrell T, Dusch V, Ethelberg S, Harris J, Hjertqvist M, Jourdan-da Silva N, Koller A, Lenglet A, Lisby M and Vold L, 2010. Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. *Euro Surveill*, 15.
- Wheeler C, Vogt TM, Armstrong GL, Vaughan G, Weltman A, Nainan OV, Dato V, Xia G, Waller K, Amon J, Lee TM, Highbaugh-Battle A, Hembree C, Evenson S, Ruta MA, Williams IT, Fiore AE and Bell BP, 2005. An outbreak of hepatitis A associated with green onions. *N Engl J Med*, 353, 890-897.
- Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ and Roderick PJ, 1999. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. *British Medical Journal*, 318, 1046-1050.
- Wichmann O, Schimanski S, Koch J, Kohler M, Rothe C, Plentz A, Jilg W and Stark K, 2008. Phylogenetic and case-control study on hepatitis E virus infection in Germany. *J Infect Dis*, 198, 1732-1741.
- Wilkinson N, Kurdziel AS, Langton S, Needs E and Cook N, 2001. Resistance of poliovirus to inactivation by high hydrostatic pressures. *Innovative Food Science & Emerging Technologies*, 2, 95-98.
- Xerry J, Gallimore CI, Iturriza-Gomara M, Allen DJ and Gray JJ, 2008. Transmission events within outbreaks of gastroenteritis determined through analysis of nucleotide sequences of the P2 domain of genogroup II noroviruses. *J Clin Microbiol*, 46, 947-953.
- Xie Y, Hajdok C, Mittal GS and Warriner K, 2008. Inactivation of MS2 F(+) coliphage on lettuce by a combination of UV light and hydrogen peroxide. *Journal of Food Protection*, 71, 903-907.
- Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y and Okamoto H, 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol*, 84, 2351-2357.
- Zakhour M, Maalouf H, Di Bartolo I, Haugarreau L, Le Guyader FS, Ruvoen-Clouet N, Le Saux JC, Ruggeri FM, Pommepuy M and Le Pendu J, 2010. Bovine Norovirus: Carbohydrate Ligand,

- Environmental Contamination, and Potential Cross-Species Transmission via Oysters. *Appl Environ Microbiol*, 76, 6404-6411.
- Zhang K and Farahbakhsh K, 2007. Removal of native coliphages and coliform bacteria from municipal wastewater by various wastewater treatment processes: implications to water reuse. *Water Res*, 41, 2816-2824.
- Zhao C, Ma Z, Harrison TJ, Feng R, Zhang C, Qiao Z, Fan J, Ma H, Li M, Song A and Wang Y, 2009. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *J Med Virol*, 81, 1371-1379.
- Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI and Monroe SS, 2006. Norovirus classification and proposed strain nomenclature. *Virology*, 346, 312-323.

APPENDICES

A. FOOD CONSUMPTION DATA

Table 11: Consumption statistics for berries and small fruits, water molluscs and crustaceans from the “Comprehensive European Food Consumption Database”

Country	Number of subjects	Berries and small fruits (grams/day)				Water molluscs (grams/day)				Crustaceans (grams/day)			
		% consumers	Mean	SD	95 th percentile	% consumers	Mean	SD	95 th percentile	% consumers	Mean	SD	95 th percentile
Austria	2123	25%	22.6	77.1	125.0	0.2%	0.2	6.7	0.0	0.6%	0.4	5.3	0.0
Belgium	3245	14%	11.3	37.9	87.4	3.3%	1.7	12.0	0.0	10.4%	2.8	12.8	20.0
Bulgaria	1204	4%	6.4	38.1	0.0	0.2%	0.2	6.1	0.0	0.0%	0.0	0.0	0.0
Bulgaria II	1723	7%	3.6	17.1	25.5	0.0%	0.0	0.0	0.0	0.0%	0.0	0.0	0.0
Czech Republic	1751	13%	6.3	25.1	46.0	0.0%	0.0	0.0	0.0	0.1%	0.1	2.2	0.0
Denmark	4118	36%	8.3	19.9	43.7	0.4%	0.1	2.4	0.0	43.5%	1.8	4.8	11.6
Estonia	1866	11%	19.9	87.8	150.0	0.1%	0.0	0.7	0.0	2.0%	0.8	8.6	0.0
Finland	2038	53%	33.2	53.4	137.8	0.7%	0.1	2.4	0.0	3.2%	0.7	6.4	0.0
France	4079	23%	10.5	30.8	59.7	14.1%	2.0	6.6	15.0	24.7%	1.3	3.6	8.6
Germany	13926	14%	14.2	47.3	105.5	0.7%	0.3	5.5	0.0	2.1%	0.5	5.9	0.0
Hungary	1360	9%	6.7	30.3	50.0	0.0%	0.0	0.0	0.0	0.1%	0.0	0.7	0.0
Ireland	958	17%	2.6	11.5	14.3	2.0%	0.2	2.0	0.0	9.1%	0.8	3.3	6.3
Italy	3323	10%	3.0	12.9	23.4	19.4%	9.4	25.4	65.1	10.1%	3.9	16.0	27.7
Latvia	2070	18%	26.2	72.3	175.0	0.0%	0.0	0.0	0.0	0.2%	0.1	1.2	0.0
Netherlands	750	3%	1.3	9.0	0.0	1.3%	0.4	3.6	0.0	4.9%	1.2	9.3	0.0
Poland	4134	12%	25.1	92.3	175.0	0.0%	0.0	0.0	0.0	0.0%	0.0	0.0	0.0
Slovakia	2761	8%	14.3	63.4	100.0	0.0%	0.0	0.0	0.0	0.1%	0.1	6.0	0.0
Slovenia	410	14%	34.6	105.2	300.0	0.5%	1.1	15.4	0.0	0.2%	0.3	6.8	0.0
Spain	418	13%	9.1	29.5	71.3	22.7%	9.7	28.0	59.3	17.9%	4.1	13.6	20.0
Spain II	1068	13%	6.3	19.6	47.5	42.1%	11.8	22.8	58.3	33.2%	5.3	11.2	30.0
Sweden	1210	27%	6.9	21.3	34.3	0.7%	0.1	1.9	0.0	23.7%	4.2	9.9	25.7
United Kingdom	1724	25%	7.0	18.3	42.3	3.4%	0.5	3.9	0.0	23.7%	2.6	7.1	16.2

Table 12: Basic information on the dietary surveys included in the “Comprehensive European Food Consumption Database

Country	Name of the dietary survey (Acronym)	Institution providing the data	Reference publication
Austria	Austrian Study On Nutritional Status (ASNS)	Institute of Nutritional Sciences - University of Vienna	(Elmadfa et al., 2009)
Belgium	Diet National 2004	Scientific Institute of Public Health	(De Vriese et al., 2005)
Bulgaria	National Survey Of Food Intake And Nutritional Status	National Centre of Public Health Protection	Not available
Bulgaria II	NUTRICHILD	National Centre of Public Health Protection	Not available
Czech Republic	SISP04	National Institute of Public Health	(Ruprich et al., 2006)
Denmark	Danish Dietary Survey	National Food Institute, Technical University of Denmark	(Lyhne et al., 2005)
Estonia	NDS 1997	National Institute for Public Health Development	(Pomerleau et al., 1999)
Finland	FINDIET 2007	National Public Health Institute - Department of Nutrition	(Paturi et al., 2008)
France	INCA2	French Food Safety Authority (AFSSA)	(AFSA, 2009)
Germany	National Nutrition Survey II	Bundesforschungsinstitut für Ernährung und Lebensmittel (Max Rubner-Institut)	(Krems et al., 2006)
Hungary	National Repr Surv	Hungarian Food Safety Office	(Rodler et al., 2005)
Ireland	NSFC	Food Safety Authority of Ireland	(Kiely et al., 2001)
Italy	INRAN-SCAI 2005–06	National Research Institute for Food and Nutrition (INRAN)	(Leclercq et al., 2008)
Latvia	EFSA_TEST	Food Centre Food and Veterinary Service of Latvia	(Šantare et al., 2008)
Netherlands	VCP2003	National Nutrition Centre	(Ocké et al., 2005)
Poland	IZZ-FAO-2000	National Food and Nutrition Institute	(Sekula et al., 2004)
Slovakia	SK MON 2008	Food Research Institute	Not available
Slovenia	CRP-2008	National Institute of Public Health of Slovenia	Not available
Spain	AESAN	Universidad Complutense de Madrid	(Requejo Marcos et al., 2002)
Spain II	AESAN-FIAB	Universidad Complutense de Madrid	Not available
Sweden	RIKSMATEN 1997-98	Swedish National Food Administration	(Becker and Pearson, 1998)
United Kingdom	National Diet & Nutrition Survey (NDNS)	Food Standards Agency (FSA)	(Swan, 2004)

Table 13: Target population, survey period, sampling design and response rate

Country	Target population	Survey period	Sampling method and sampling frame	Sample unit	Response rate (%)
Austria	Adults	May '05 - February '06	Random from telephone book, Job centres, gynaecologists, university	Individual	48
Belgium	Adults	February '04 - February '05	Random from the general population census	Individual	35
Bulgaria	Adults	March '04 - August '04	Random from the national population register	Individual	85
Bulgaria II	Small children	April '07 - August '07	Random from the register of general practitioner's practices	Individual	78
Czech Republic	Children and adults	November '03 - November '04	Random from the address register	Household	54
Denmark	Children and adults	June '00 - December '02	Random from the national population register	Individual	53
Estonia	Adults	March 1997 - September 1997	Random from the national population register	Individual	67
Finland	Adults	January '07 - March '07	Random from the national population register	Individual	62
France	Children and adults	December '05 - April '07	Random from the general population census	Household	60
Germany	Adults and adolescents	November '05 - January '07	Random from the national population register	Individual	42
Hungary	Adults	October '03 - December '03	Random from the general population census	Individual	27
Ireland	Adults	October 1997 - October 1999	Random from the electoral list	Individual	63
Italy	Children and adults	October '05 - December '06	Random from the telephone book	Household	33
Latvia	Children and adults	June '08 - November '08	Random from a consumer panel	Individual	56
Netherlands	Adults	October '03 - December '03	Random from a consumer panel	Individual	42
Poland	Children and adults	September '00 - November '00	Random from the sample of the household budget survey	Household	96
Slovakia	Adults	January '08 - December '08	Random among employees of confectionary and bakery manufactures and canteen	Individual	98
Slovenia	Adults	September '07 - April '08	Random from the national population register	Individual	52
Spain	Adults	January 1999 - November '01	Random from the university, health centre, pharmacies	Individual	71
Spain II	Adults	January '09 - September '09	Random from the university, health centre, pharmacies	Individual	28
Sweden	Adults	January 1997 - January 1998	Random from the national population register	Household	60
United Kingdom	Adults	June '00 - June '01	Random from the postcode address file	Household	61

B. RASFF NOTIFICATIONS

Date	Reference	Notifying Country	Reason	Virus	Product	Origin	Harvesting date	Distribution	Amount kg	Consignm ent date	Action	Reported cases	International response
Mar-10	10-600	Netherlands	food poisoning outbreak	HAV	sun dried tomatos and related products	Germany, France, Italy, Turkey	NR	Netherlands	NR	NR	Withdrawal from the market	13	controls in Greece
Mar-10	2010.0321 2010.0322 2010.0324	Denmark	food poisoning outbreak	NV	oysters "Normandie", "Isigny", "Utah"	France	NR	Denmark	126kg net weight of lot	NR	Product consumed	23	NR
Feb-10	2010.0199	Ireland	food poisoning outbreak	NV	Oysters	Ireland	NR	Ireland, UK	NR	NR	Withdrawal from the market/ press release	NR	NR
Feb-10	2010.0191	Ireland	food poisoning outbreak	NV	oysters	Ireland	22-Jan-10	Ireland, UK	417 kg gross weight of the lot	NR	Withdrawal from the market/ press release	4	NR
Feb-10	2010.0163	Norway	food poisoning outbreak	NV	oysters	France	NR	UAE, Hong Kong, Singapore, Thailand, Norway, Netherlands	1960 gross weight of the lot	Jan-10	Product recall	37	NR
Feb-10	09-580-add01	France	food poisoning outbreak	HAV	sun dried tomatos and related products	Turkey	NR	Switzerland, Luxembourg, Spain, Belgium, Italy, Germany, France, Netherlands, Greece, USA, Australia, Israel, Poland	104120kg	NR	Withdrawal from the market	>43	NR
Jan-10	2010.0081	Denmark	food poisoning outbreak	NV	lettuce	France	NR	Denmark, Norway, France, Germany		NR	Product recall/ press release	>260	NR
Jan-10	2010.ACL	France	border rejection	NV	frozen scallops	Peru	NR	France	20433 total net weight of the lot	NR	Re-export	NR	NR
Nov-09	2009.1620	Denmark	food poisoning	NV	frozen raspberries	Serbia Belgium	June-July/09	Denmark	5800 kg gross weight of the lot	NR	Withdrawal from the market Public warning/press release	6	NR
Nov-09	09-580-add02	Australia	food poisoning outbreak	HAV	sun dried tomatos and related products	unknown	NR	NR	NR	NR	Recall	>250	NR
Oct-09	2009.1371	Finland	food poisoning	NV	frozen raspberries	Poland	NR	Finland	20,160 kg net weight of the lot	NR	Withdrawal from the market	>100	NR
Oct-09	2009.1361	Sweden	consumer complaint	NV	frozen raspberries	Serbia	NR	Sweden	NR	NR	Nr	19	NR
Oct-09	2009.1380	Belgium	official control on the market	HAV	mussels	Netherlands	NR	Belgium	NR	NR	No stock left- no action	NR	NR

Date	Reference	Notifying Country	Reason	Virus	Product	Origin	Harvesting date	Distribution	Amount kg	Consignm ent date	Action	Reported cases	International response
Oct-09	2009.1382	Belgium	official control on the market	HAV	mussels	Netherlands	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Oct-09	2009.1383	Belgium	official control on the market	HAV	mussels	Netherlands	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Oct-09	2009.1384	Belgium	official control on the market	HAV	mussels	Netherlands	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Oct-09	2009.1385	Belgium	official control on the market	HAV	mussels	Netherlands	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Oct-09	2009.1386	Belgium	official control on the market	HAV	living bivalve molluscs	France	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Oct-09	2009.1387	Belgium	official control on the market	HAV	clams	France	NR	Belgium	NR	NR	No stock left- no action	NR	NR
Jul-09	2009.0854	Finland	food poisoning	NV	frozen raspberries	Poland	NR	Finland	19060 kg	NR	Withdrawal from the market	100-150	NR
Jun-09	2009.BEQ	France	border rejection	NV	frozen scallops	Peru	NR	France	20980 kg net weight of lot	NR	Border rejection-re-export	NR	NR
Jun-09	2009.0732	Finland	food poisoning	NV	frozen raspberries	Poland	2008	Finland	18270 kg gross weight of lot	NR	Withdrawal from the market	20	NR
Mar-09	2009.0340	Norway	food poisoning	NV	oysters	Sweden	NR	Norway	NR	NR	Withdrawal from the market	19	NR
Sep-08	2008.1153	Norway	official control on the market	NV	oysters	Ireland	NR	Norway	2400 oysters	NR	Withdrawal from the market	NR	NR
Sep-08	2008.1079	Spain	official control on the market	HAV	tellina	Peru	NR	France, Belgium, Netherlands, Denmark, Portugal, Italy, Germany, Spain, Austria	NR	NR	Withdrawal from the market; suspension of the export; closure of all the zones and area of extraction and of the production plants of bivalves molluscs on the Peruvian coast-line	5	NR
Apr-08	2008.0448	Norway	official control on the market	NV	oysters	United Kingdom	NR	Norway	1600 oysters	NR	Sales ban	NR	NR
Apr-08	2008.0421	Norway	food poisoning outbreak	NV	oysters	United Kingdom	NR	Norway	1200 oysters	NR	Sales ban	ca 6	NR
Apr-08	2008.0380	Italy	company's own check	HAV	oysters	France	29/02/08 and 06/03/08	Italy	ca 730	packaging 11/03/2008	Withdrawal from the market	NR	NR
Mar-08	2008.0322	Norway	food poisoning outbreak	NV	oysters	United Kingdom	NR	Norway, UK	1600 oysters	NR	Nr	ca 6	NR
Jan-08	2008.0078	France	food poisoning	NV	oysters	Spain	NR	France	110 kg	01/12/2007	No stock left	several cases of collective food poisoning	NR

Date	Reference	Notifying Country	Reason	Virus	Product	Origin	Harvesting date	Distribution	Amount kg	Consignm ent date	Action	Reported cases	International response
Jan-08	2008.0086	Netherlands	consumer complaint-food poisoning	NV	cupped oysters	France	Dec-07	Netherlands	NR	production date 09/01/08	Recall	6	NR
Jul-07	2007.BVQ	United Kingdom	consumer complaint	NV	oysters	United Kingdom	NR	UK, Hong Kong, Switzerland, Germany	Hong Kong-138 kg Switzerland-36 kg	04-16/07/07	Withdrawal from sale	> 80	NR
Jan-07	2007.0021	Malta	consumer complaint	NV	raw oysters	France via Italy	NR	Malta, Italy	to Italy 1050 kg to Malta- 270 kg	NR	Reporting country-product recall, media advert, product destroyed	ca 70	NR
Aug-06	2006.0546	Netherlands	consumer complaint	NV	frozen raspberries	Chile via Germany	NR	Netherlands	23520 kg	20/04/2006	Remaining stock blocked	42-45	NR
Aug-06	2006.0551	Sweden	consumer complaint	NV	frozen raspberries	China via Denmark	NR	Sweden	11205 kg	NR	Product recall	43	NR
Apr-06	2006.0236	Germany	official control on the market	NV	oysters	France	NR	Austria, Belgium, Switzerland, China, Czech Republic, Germany, Hungary, Italy, Russia, Slovakia	NR	NR	Product withdrawal	NR	NR
Mar-06	2006.0211	Netherlands	official control on the market	NV	living oysters	France	NR	Netherlands, Italy	NR	NR	Product detained	NR	NR
Mar-06	2006.0182	Norway	consumer complaint	NV	raw oysters	France	NR	Norway	386 kg net weight of lot	13/02/2006	No stock left	2	NR
Mar-06	2006.ASW	Denmark	food poisoning outbreak	NV	frozen raspberries	Serbia and Montenegro via the Czech Republic	NR	Denmark	20160kg net weight of the lot	NR	Product withdrawal	25	NR
Mar-06	2006.ASI	Netherlands	food poisoning outbreak	NV	raw oysters	France	NR	Netherlands	NR	NR	Product blocked, withdrawal by the producer	3	NR
Mar-06	2006.0163	Italy	food poisoning outbreak	NV	raw oysters	France	NR	Italy	NR	NR	No stock left	12	NR
Mar-06	2006.0159	Denmark	food poisoning outbreak	NV	live oysters	France	NR	Denmark, Austria, Germany, Dubai	640kg net weight of the lot	NR	No stock left	46	NR
Mar-06	2006.0162	Italy	food poisoning outbreak	NV	raw oysters	France	NR	Italy	900 kg net weight of the lot	NR	Resoaking of the batch	6	NR
Mar-06	2006.0158	Denmark	food poisoning outbreak	NV	live oysters	UK	NR	Spain, Netherlands, Denmark, France	100kg net weight of the lot	NR	Product destroyed Administrative penalties to the establishment	>25	NR
Jan-06	2006.AGH	Italy	official control on the market	HAV	oysters	France	NR	Italy	NR	NR	No stock left- no action	NR	NR

Date	Reference	Notifying Country	Reason	Virus	Product	Origin	Harvesting date	Distribution	Amount kg	Consignm ent date	Action	Reported cases	International response
Sep-05	2005.653	Denmark	Isolated from patents	NV	frozen raspberry	Poland	NR	Austria, Switzerland, Czech Republic, Germany, Denmark, Finland, France, Israel, Lithuania, Latvia, Netherlands, Russia, Sweden, Slovakia, USA	5040 kg	various	Heat treatment + info request	33	Lithuania-product destroyed or returned to manufacturer
Jun-05	2005.386	Denmark	Illness	NV	Frozen raspb.	Poland	NR	Denmark	19.92	20/01/2005	Recall	>350	Germany
Sep-04	2004.CBU	Italy	Detection in food	HAV	Live oysters	France	NR	Italy	750 kg net weight	NR	Nr	NR	Investigation of bank
Sep-03	2003.BUO	Sweden	Illness	calicivirus	Frozen raspb.	Serbia and Montenegro	2002	NR	NR	NR	Local recall	>50	None
Nov-02	2002.BLM	Italy	Detection in food	HAV	Oysters	France	NR	Italy, France	NR	NR	Product seized	NR	NR
Oct-02	2002.BFJ	Italy	Presence in food	HAV	Live oysters	France	702	NR	NR	Feb-10	Recall	NR	Request for information. No response
Feb-02	2002.060	Ireland	Illness and virus presence in food	NV	Live oysters	Ireland	various	UK, Hong Kong	NR	various	Alert	NR	Ireland announced formal closure of site
Feb-02	2002.059	Ireland	Presence of virus in food	NV	Live oysters	Ireland	202	France	NR	NR	Formal closure of site	NR	
Jul-01	2001.IV	Spain	Detection in product	HAV	Clams	France	03 and 10-Jul-01	Spain	150	Jan-10	None, already consumed	NR	None
Feb-01	2001.022	Netherlands	Illness	NV	Live oysters	France	NR	Belgium, Luxembourg, Netherlands	NR	NR	Recall	>13	None
Feb-01	2001.CI	Finland	Illness and virus detection	calicivirus	Live oysters	France	101	NR	170	Jan-10	Recall subsequent batch	8	None
Jul-00	2000.GP	Spain	Detection in food	HAV	Frozen scallops	Peru	NR	NR	1376	NR	Import stop	NR	None
Jun-00	2000.FO	Spain	Detection in food	HAV	Wedge shell	Peru	NR	NR	24000kg	NR	Import stop	NR	None

C. REVIEW OF LEGISLATION

Hygiene package

Regulation (EC) No 852/2004 on the hygiene of foodstuffs provides general rules for food business operators for the production and processing of all food throughout the food chain. General implementation of procedures is based on the HACCP principle, together with application of good hygiene practice. Provisions for primary production cover e.g. the transport, storage and handling of primary products at the place of production and transport to an establishment. According to Regulation guides to good practice should be developed to encourage the use of appropriate hygiene practices at farm level. Guides may include e.g. the use of water, organic waste, the proper disposal of waste, protective measures to prevent introduction of contagious diseases transmissible to humans through food, procedures, practices and methods to ensure that food is produced, handled, stored and transported under appropriate hygienic conditions etc.

Regulation (EC) No 853/2004 laying down specific hygiene rules for food of animal origin provides specific hygiene requirements for the production and harvesting of live bivalve molluscs. Health standards for bivalve molluscs include microbiological criteria, organoleptic characteristics and marine biotoxins. No special reference on viruses is set in this legislation.

Use of water

The provision for food business operators to possibly use clean water is referred to in several parts of the hygiene Regulations. For producing or harvesting plant products or producing primary products of animal origin the water used has to be potable or clean whenever necessary. Clean water shall not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the health quality of food.

Fishery products and live bivalve molluscs

Where fish or live bivalve molluscs are handled as a part of the primary production, potable water or clean water (clean seawater or fresh water of similar quality) shall be used to prevent contamination. Similar provisions are laid down in respect of the handling of fishery products or live bivalve molluscs when not a part of the primary production.

Both in primary production and in further handling of fishery products or live bivalve molluscs after primary production, measures must be taken to ensure that the clean water used is not a source of contamination for the fishery product or live bivalve molluscs. Procedures to monitor and document the safety/quality of the water must be put in place by operators. These measures must be included in the HACCP-based procedures, when clean water is to be used at any stages after primary production. It is the task of the competent authority to verify whether the procedures developed by the operators are sufficient and carried out properly and do not pose a risk to consumers.

When deciding to use such water and/or when developing procedures based on the HACCP principles, food business operators should pay attention to different aspects, such as:

- Studying the composition of the water (including possible contaminants, e.g. chemical, microbiological, toxic algae, etc) at the intake water point and its possible variations (seasonal effects, rainfall dependent discharges, etc) to ensure that it does not contain micro-organisms, harmful substances or toxic marine plankton in quantities capable of directly or indirectly affecting the safety of the food,
- Assessing the impact of natural or man-made contamination sources and the possible protective measures to address them (a river mouth, dredging operations, etc.),
- Describing the water production (reclamation, treatment, etc), storage and distribution systems.

When making use of such water, food business operators must ensure that it is not a source of contamination for fishery products or live bivalve molluscs. There are different means of reaching this objective, such as:

- Pumping water for the production of clean water from a position that avoids contamination of the water supply, avoiding polluted areas, pumping water in depth, pumping water in remote areas). This may be enough for vessels operating in open water.
- Using a water treatment system to ensure that the requirements for clean water are met. This may involve a particle retention step, followed by an adsorption step and a sanitising step, and/or
- Other appropriate procedures.

Guides to good practices may be appropriate tools to assist food business operators in defining these means so as to ensure that clean water used is not a source of contamination for the fishery product.

General food law

Article 14 on food safety requirements of Regulation (EC) No 178/2002 of the European Parliament and of the Council laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, states that unsafe food shall not be placed on the market. Unsafe food is considered to be food that is injurious to health or unfit for human consumption. According to the article food that complies with specific Union provisions, like microbiological criteria set in Regulation 2073/2005, is deemed to be safe.

However, as prescribed in Article 14 (8) of the Regulation, the competent authorities of Member States may take appropriate measures to impose restrictions where there are reasons to suspect, on a case-by-case basis, that despite conformity with the Union legislation, the food in question is unsafe. Moreover, in case of emergency, or if official control analysis reveals that a foodstuff is likely to constitute a serious risk to human health, the procedure set out in Article 54 of Regulation 178/2002 could also apply and national measures could be adopted on an interim basis.

Animal by-products

Regulations (EC) No 1774/2002 laying down health rules concerning animal by-products not intended for human consumption and (EC) No 181/2006 on organic fertilisers and soil improvers other than manure lay down health rules on the classification of manure (excrements and urine from farmed animals) and the possibilities to apply it to land, as well as on the production, placing on the market and use of organic fertilisers which have been produced from animal by-products. Furthermore, Regulation (EC) No 1774/2002 lays down rules for the transformation of animal by-products into biogas and for their composting.

Waste

Article 13 of Directive 2008/98/EC on waste states on protection of human health and the environment that Member States shall take the necessary measures to ensure that waste management is carried out without endangering human health, without harming the environment and, in particular:

- (a) without risk to water, air, soil, plants or animals;
- (b) without causing a nuisance through noise or odours; and
- (c) without adversely affecting the countryside or places of special interest.