



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Achievement V — Methods for breaking the transmission of pathogens along the food chain

Detection of viruses in food

Frederik Widén^{a,*},
Ivar Vågsholm^b, Sándor Belák^{a,c}
and Shaman Muradrasoli^b

^aThe National Veterinary Institute (SVA), VIP, Section
of Research and Development, Ulls Vag 2B, 75189
Uppsala, Sweden (Tel.: +46 18674633; e-mail:
frederik.widen@sva.se)

^bThe Swedish University of Agricultural Sciences
(SLU), Department of Biomedical Sciences and
Veterinary Public Health, Sec. Bacteriology and Food
Safety, Sweden

^cSwedish University of Agricultural Sciences (SLU),
Department of Biomedical Sciences and Veterinary
Public Health, Sec. Virology and Parasitology, Sweden

Traditionally the focus for control of food-borne disease has been bacteria. During the last decade viruses have emerged as important sources of food borne human disease. Since the traditional bacteriological indicators, are not reliable for viral contamination, new methods are needed. PCR has enhanced the detection of virus in food. A challenge for developing detection reliable methods for viruses in food is that food matrices vary in composition, high sequence variability and

inhibitors may be present. Therefore it is necessary to develop assays that have high diagnostic sensitivity, are broad and robust, and combine sample concentration and removal of inhibitors.

Introduction

Reducing the burden of food and water borne, diseases represents one of the major challenges for achieving the millennium development goals (MDG) during the next decennia. The disease burden from diarrheal diseases are 72.8 million disability adjusted life years (DALY) and responsible for constitute the second leading cause of burden of disease in the world. (WHO, 2004). Furthermore, gastroenteritis is a major cause of malnutrition in infants and young children in developing countries, and it has been reported that 1.8 million deaths in total and 17% of all deaths of children under 5 years of age can be attributed to diarrhoeal diseases (WHO, 2004, 2007). In a large proportion of these cases, the causative agent has been transmitted through the food chain, including drinking water. It has been estimated that in USA 76 and 14 million persons every year suffer from food-borne disease, of unknown and known causes respectively (Mead *et al.*, 1999; Newell *et al.*, 2010).

Furthermore, viruses that cause hepatitis, e.g. Hepatitis E (HEV), in humans and are of major importance in developing countries, and increasingly also in developed countries, may be transmitted through contaminated water or through food (Purcell & Emerson, 2008). This is also true for some viruses causing meningo-encephalitis in humans, e.g. Tick Borne Encephalitis virus (TBE) (Newell *et al.*, 2010; WHO, 2007).

Until fairly recently the importance of viruses as food-borne pathogens was not realized. There are several reasons for the late recognition of virus as important food-borne pathogens.

Most important is the failure to detect viruses in outbreaks of food borne disease. Moreover, deteriorating quality of food bacterial contamination of food may be evident as changes in organoleptic appearance as a result of growth of spoilage bacteria. However for both viruses and bacteria, contamination presenting a public health risk may not be associated with changes of the foodstuffs sensory characteristics.

High viral loads have been found in food declared safe by traditional food safety controls (Newell *et al.*, 2010).

* Corresponding author.

Moreover, indicator organisms of fecal contamination, such as number of *Escherichia coli* or coliformes, are not suitable to demonstrate freedom or presence of norovirus in food (SCVMPH, 2002).

Virus contamination of food often occurs late in the food chain when food is handled, for example in the case of noroviruses, in restaurants or in nursing homes, or in cruise-ships (ECDC, 2009). Food may become contaminated with norovirus by an infected food handler whom is not yet aware of being infected. Even with the application of highly sensitive molecular methods the detection of virus (e.g. norovirus) in food constitutes a challenge due to the low number of virus particles needed to cause disease, the high variability of the virus genome and the possible presence of inhibitory substances in such a complex and variable matrix as food. Furthermore, some food-borne infections of viral origin have a long incubation period lasting several weeks (like HEV) and therefore usually no food is available for testing once the disease is diagnosed. Furthermore, as a consequence of the difficulties mentioned above and in contrast to the situation with bacterial food-borne pathogens, there is no efficient monitoring system in place for the detection of viral contamination of food (Newell *et al.*, 2010).

The food-borne viral agents causing enteric disease are norovirus, astrovirus, rotavirus and coronavirus, of which noroviruses appears to be the most frequently detected (FAO/WHO, 2008). Hepatitis can be caused by Hepatitis A or Hepatitis E virus while encephalitis can be caused by enterovirus or by tick-borne encephalitis virus. Other viruses have also been mentioned as possible food-borne pathogens but are at present not significant (FAO/WHO, 2008; Newell *et al.*, 2010).

Virus detection by PCR enables much higher analytic sensitivity for detection of virus in food (Fong & Lipp, 2005) compared to electromicroscopy or immunodiagnostic assays (Koopmans & Duizer, 2004). This very sensitive technique frequently enables the detection of as little as 10 virus copies. Such high analytical sensitivity is a prerequisite for the detection of virus in food, because the infectious dose of several food borne viruses is in that range, but does not overcome the problem with virus being diluted or inhibited in a large food matrices. The samples that are taken and analyzed for virus contamination are much smaller (less than 0.5 g) than what is usual when testing food for bacterial contamination (25 g). Because the number of virus particles required for catching disease is very low and the virus particles are diluted in a large volume of food matrix with a very small sample being analyzed in each PCR reaction, even an extremely sensitive assay may be unable to reliably detect virus contamination. Therefore the virus particles in the food sample needs to be concentrated before analysis is performed to achieve a reasonable sensitivity of the diagnostic procedure.

Due to lack of routine or regular monitoring of foods for the presence of viral contaminants, there is relatively little information on the general occurrence of viruses in

different food matrixes. However, there are some reports on food-borne viral infections caused by consumption of contaminated food.

In this paper we review the relevant viruses, the virus survival in foodstuffs, possible routes of transmission, and discuss possible detection methods.

Food borne viruses and their survival in food

Viruses cannot replicate in food and therefore the number of infectious virions will not increase during processing and storage. Although, limited data describing the survival of virus particles on food surfaces are available, it has been reported that gastroenteric viruses can remain infective for very long time on environmental surfaces. The survival of enteric viruses in food products varies due to the stability of the virus with respect to resistance to chemical and physical agents such as drying, heat, low pH, and solvents (John & Rose, 2005). Human enteric viruses are non-enveloped viruses, and highly stable in environmental samples, such as water and different food samples. Cooking of food will normally kill the food borne viruses.

Hepatitis E Virus

Hepatitis E Virus (HEV), an equally important cause of viral hepatitis as HAV, is spread through the fecal-oral route and may be water or food borne (Purcell & Emerson, 2008). The virus is non-enveloped, belongs to the Hepeviridae family and causes acute hepatitis, clinically indistinguishable from HAV. The most common source of infection is faecally contaminated drinking water. In recent years, water borne infections have been linked to major outbreaks. HEV genotype 1 and 2 is endemic in many developing countries throughout Asia and Africa where large outbreaks have also occurred. These genotypes only infect humans, are not zoonotic and there is a strong connection between poor hygienic standards and the spread of HEV gt 1 and 2. Sporadic cases have also been associated with eating seafood contaminated by sewage. In developed countries the consumption of undercooked pig liver, pig or wild boar and deer meat constitute a source of zoonotic transmission of HEV (Tamada *et al.*, 2004; Tei *et al.*, 2004). In the latter case genotype 3 and 4 have been implicated. Recently the transmission of HEV genotype 3 from pigs to humans through lightly smoked sausage containing pig liver and pork was described (Colson *et al.*, 2010). Genotype 3 is present in the pig population world-wide while genotype 4 is present in the pig population of East and South-East Asia. The virus is almost as resistant to environmental influences as HAV. It has been demonstrated that high pressure treatment of food efficiently inactivates feline calicivirus and murine norovirus that was used as model viruses for HEV inactivation (Widén, 2009).

Tick Borne Encephalitis Virus (TBEV)

This virus belongs to the family of Flaviviridae, genus flavivirus and is an enveloped RNA virus and thereby

sensitive to the environmental conditions. The virus is spread by ticks that can infect humans. An alternative, less common, mode of transmission through consumption of goat, sheep or cows milk has been described in Eastern and Central Europe and has been estimated to 5% of all cases in countries where this transmission occurs (Cisak *et al.*, 2010; Dumpis, Crook, & Oksi, 1999; Kerbo, Donchenko, Kutsar, & Vasilenko, 2005; Sumilo *et al.*, 2008).

Furthermore it has been demonstrated that goats do shed TBE virus in milk after experimental infection and that pasteurization of the milk or immunization of the goats efficiently protects consumers from infection (Balogh *et al.*, 2011). Survival time for TBEV in milk is not known but is at least 12 days when kept in refrigerator (Holzmann *et al.*, 2009).

Noroviruses

It has been estimated that two thirds of the cases of food-borne disease in USA can be attributed to norovirus (NoV), also referred to as Norwalk or calici viruses, and in the United Kingdom the cost of infections with this agent has been estimated to 24 million pounds per year affecting more than 600 000 persons annually (Anonymous, 2000; Mead *et al.*, 1999). The most common cause of nonbacterial gastroenteritis worldwide is NoV (FAO/WHO, 2008; Hee-Yeon Kim, In-Gyun, & GwangPyo, 2008) and it has been suggested that the global incidence of NoV associated disease is increasing. The virus belongs to the Caliciviridae family. The worldwide spread of NoV is believed to be associated to the development of the food market with large worldwide shipments of fresh food, in addition with moderate but inadequate improvements of hygiene control (Koopmans, 2005). This hypothesis is based on the assumption that food-borne illnesses are emerging (Newell *et al.*, 2010), and NoV is the most common cause (Mormann, Dabisch, & Becker, 2010). Foodstuffs that are commonly implicated in NoV outbreaks are raw or poorly cooked seafood and meat, vegetables and fruits as well as ready-to-go food (FAO/WHO, 2008; Kim *et al.*, 2008) g. NoV has been detected in fresh fruits, vegetables, berries, oyster and mussels. During the last decade several NoV outbreaks have been linked to consumption of raspberries (Le Guyader *et al.*, 2004). Outbreaks associated with consumption of raw oysters and raspberries have been found in several European countries and published in *Eurosurveillance*, (Maunula *et al.*, 2009; Westrell *et al.*, 2010) However, any type of food product is potentially a vehicle for NoV transmission because it may become contaminated by an infected food handler (Hee-Yeon Kim *et al.*, 2008). This virus endures temperatures up to 60 °C (Glass, Parashar, & Estes, 2009), and mild pasteurization at 65 °C, used for raspberry puree, may be insufficient if the viral load is high (Baert, Uyttendaele, Van Coillie, & Debevere, 2008). Furthermore the virus remains stable at pH below 3. The virus is resistant to environmental influences and it

has been reported to survive for at least 12 days on dry surfaces.

Hepatitis A (HAV)

Hepatitis A virus belongs to Picornaviridae, and is an important cause of viral hepatitis that is excreted in faeces and can be transmitted through contaminated food or water between humans only. This virus has been implicated in very large outbreaks, for example 1988 in Shanghai, China where 250.000 fell ill due to this virus (Halliday *et al.*, 1991). More recently, 1600 laboratory confirmed cases occurred in Czech Republic and 2800 in Latvia, during 2008 (Castkova & Benes, 2009; Perevoscikovs *et al.*, 2009). HAV is also relatively resistant to many environmental conditions, physical and chemical agents (Mbithi, Springthorpe, Boulet, & Sattar, 1992), and may retain their infectivity for days to weeks in dried feces (McCausland, Bond, Bradley, Ebert, & Maynard, 1982). Because of the absence of envelope the virus is very stable. It has been shown that the virus can survive freezing and pasteurization (Koopmans & Duizer, 2004). It can remain infectious in fresh water or sea water for up to 12 months and accumulate in oysters and mussels that filter the sea water. HAV can survive on surfaces for more than 60 days and on hands more than 4 h. Food can be contaminated with HAV in three main ways; at the point of sale, contamination of food during cultivation, harvesting, processing, or distribution or by exposure to contaminated water (Fiore, 2004). Apart from the indirect transmission through contaminated food the direct transmission from person to person is the most important route. Recent reports show that HAV-infected food handlers at the point of sale (e.g. restaurants) or catering constitute the most frequent source of food-borne hepatitis A outbreaks (Fiore, 2004). Although, consumption of improperly cooked shellfish continues to be the major source of HAV food-borne associated outbreaks, many types of berries, such as blueberries, strawberries and raspberries has also been implicated as a source of HAV outbreaks (Calder *et al.*, 2003). Niu *et al.*, 1992, reported investigation of a multistate outbreak of HAV associated with frozen strawberries involving at least 262 persons in 5 US states.

Enteroviruses

Enteroviruses belong to the Picornaviridae family and are found in aquatic environments and are transmitted by contaminated food or water *via* the fecal-oral route, as hepatitis A virus and noroviruses (Schwab, De Leon, & Sobsey, 1995). Food-borne transmission of enterovirus with berries has been documented. Enterovirus infected persons may suffer from meningitis or encephalitis but other organs may also be affected. Enteroviruses are characterized by their stability to acidic conditions, both in the gastrointestinal tract and in the environment. Virus is excreted in large numbers in stool during an infection and can remain infectious in the environment for several days to weeks. Although the main transmission route is fecal-oral

transmission, contaminated food and water are major sources of infection (Kocwa-Haluch, 2001).

Rotavirus

Rotaviruses are non-enveloped double stranded RNA viruses that belong to the Reoviridae family. There are 7 serotypes of rotavirus (A–G). The rotaviruses infect both respiratory tract and gastrointestinal tract, via fecal-oral route and person to person contact, and are a dominant etiologic agent of severe diarrhea in children. Viral particles are shed in feces in large number during an infection, and can contaminate surface water, groundwater, drinking water and food (He *et al.*, 2008; Meleg *et al.*, 2008; Rutjes, Lodder, van Leeuwen, & de Roda Husman, 2009; Steinmann, 1981).

According to estimates from WHO more than a million children die each year from rotavirus infection, due to severe dehydration (WHO, 2009). In recent years, reports have shown that the incidence of rotavirus disease is similar in both industrialized and developing countries (Parashar, Hummelman, Bresee, Miller, & Glass, 2003).

The virus is very stable in the environment, resistant to most disinfectants and can survive long time, up to weeks on contaminated surfaces. Apart from the direct route from person to person the virus can be transmitted indirectly through ingestion of contaminated food and drinking water. Ready-to-eat food like prepared salads, sandwiches, and other foods that require no cooking before consumption can easily be contaminated by infected food handlers. Rotavirus outbreaks have frequently been associated with consumption of contaminated drinking water and oysters (Kittigul *et al.*, 2008; Meleg *et al.*, 2008).

Routes of contamination

Most of the virus contamination of food occurs through contact with sewage, contaminated water used for irrigation, washing, food processing or from an infected person who has handled the food during the production or processing (SCVMPH, 2002). In the latter case the personal hygiene of food handler is of great importance. Contaminated surfaces are also an important source of contamination. Food products may be contaminated at the source, usually through contact with contaminated irrigation water or sewage contaminated sea water. Shellfish, oysters and mussels that are particularly prone to contamination from sea water mixed with effluents from urban areas because of their ability to concentrate virus and are therefore often involved in outbreaks worldwide. Fruits, berries and lettuce are often contaminated during harvesting, handling and preparation by HAV or NoV infected workers (SCVMPH, 2002; WHO, 2007). In the case of HEV and TBE the product is contaminated by infection of the live animal. In this manner the liver of pigs may become infected with HEV. Other tissues, e.g. kidney, may become infected in the same manner or contamination of the meat may occur during slaughter. Milk and milk

products originating from infected goats, sheep or cows may contain infectious TBE virus (see above).

Review of possible detection methods

Hepatitis E virus

In spite of intensive trials it has proven very difficult to grow HEV in cell culture. The successful and continuous replication of HEV *in vitro* in PLC/PRF/5 A549 cell lines was published by one group (Okamoto, 2011; Tanaka *et al.*, 2009) while another group reported successful *in vitro* replication in PSI and CLAB cell line (Cencic, 2008). Other groups have published temporary low level replication of HEV (Emerson, Arankalle, & Purcell, 2005). While this constitutes an important step forward for the detection and studies of HEV, cell culture for HEV is still not a tool generally available to the scientific community or food safety laboratories. Several PCR and real-time PCR protocol for HEV detection has been published. Real-time PCR is suitable for screening of samples and detect genotypes 1, 2, 3 and 4 with high sensitivity (Gyarmati *et al.*, 2007). This PCR is also suitable for testing various food samples of porcine origin (data not shown). To determine the genotype a gelbased PCR amplifying part of the RNA polymerase gene can be used (Zhai, Dai, & Meng, 2006).

TBE virus

Contamination of milk with TBE virus may be detected through RT-PCR, virus isolation on cell culture, inoculation of suckling mice or indirectly through antibody detection with serological assays. The replication of TBE virus in a pig intestinal and macrophage functional cell model has been reported (Cencic & Avsic-Zupanc, 2009).

Norovirus

Due to lack of appropriate growth conditions in a cell culture system, (NoVs) are detected mostly by molecular methods (Patel, Hall, Vinje, & Parashar, 2009). However, traditional detection methods such as electron microscopy (EM) and antigen detection assays (e.g. ELISA) are still frequently used for detection of NoV in human stool samples. Before the time of molecular biology techniques electron microscopy (EM) was for a long time the only method to detect NoV (Caul & Appleton, 1982). The advantages of EM is that it allows visualization of characteristic viral morphology, it is rapid and requires minimal sample preparation and have therefore sustained to be a valuable diagnostic tool for NoV infection. However, there is some disadvantages, the technique requires samples with large number of virus particles (e.g. low sensitivity), highly skilled personnel and well equipped laboratory, and can only be used for detection of NoV in clinical samples and not environmental- and food samples.

Commercial immunoassays for detecting norovirus are available. These can be used close to the patient or the outbreaks but suffer from serious limitations regarding

sensitivity and specificity. Therefore the use of such assays should be combined with other more reliable tests.

Currently the most commonly used method to detect and differentiate NoV is RT-PCR assays. Several real-time RT-PCR assays have been described for NoV detection in clinical samples during the last decade. The very high sequence variability of norovirus constitutes the most serious obstacle for PCR assays for norovirus detection. As a result a wide range of sequence variants exists and new ones a constantly evolving. The ideal test should thus be able to detect the broad range of norovirus variant that exists and as well as new variants. Most of the assays target the open reading frame 1 (ORF1) of the RNA-dependent-RNA-polymerase (RdRp) gene, because it is well conserved among the different NoV (Green, Gallimore, Norcott, Lewis, & Brown, 1995; Haffiger, Gilgen, Luthy, & Hubner, 1997; Jiang *et al.*, 1999; Nakayama *et al.*, 1996; Vinje & Koopmans, 1996). Katayama *et al.* showed that the ORF1-ORF2 junction is well conserved and suitable for real-time TaqMan assays (Katayama *et al.*, 2002). Assays based on this region have also been developed by other groups (Hohne & Schreier, 2004; Jothikumar *et al.*, 2005). This assay is able to detect a wide range of noroviruses from genogroup 1 and 2 with an ability to detect as little as 100 genomic equivalents. Commercial PCR assays are also available. However, a comparison of two commercial kits with the assay proposed by CEN/TC/WG6/TAG4 group showed that the commercial kits had a much poorer performance (Butot *et al.*, 2010).

There is no threshold for infectivity established 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 to 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.*, 2010; Le Guyader *et al.*, 2008; Lowther, Avant, Gizynski, Rangdale, & Lees, 2010). However, a correlation has been found between the numbers of NoV genome copies in oysters, and the amount of self reported illness in a specific study in the UK.

Hepatitis A virus

Detection of HAV in shellfish with assays based on PCR has been reported since the mid nineties. Currently, there are numerous publications demonstrating the development and performance of PCR-based detection methods for HAV in shellfish (David Lees., 2010).

For diagnostic use on clinical samples, other detection methods such as immunoassays are available; however they are not suitable for environmentally contaminated samples such as bivalve shellfish or other foodstuff. A Technical Advisory Group (TAG), consisting of selected European laboratories has since 2004 worked on recommendations for the development of standard methods for detection of noroviruses and HAV in food (including

shellfish). This work is carried out on behalf of the European Committee for Standardization (CEN). A series of studies on the method and the different stages of development of the method are evaluated by inter-laboratory comparisons. The methods are under development and evaluation by TAG members and a reference method is expected to be available in the form of a scientific publication, year 2012 (David Lees., 2010).

Several groups have demonstrated NoV findings in food samples by RT-PCR. Maunula *et al.* could detect NoV GI was in 3/5 raspberry samples analyzed by NoV real-time RT-PCR (Maunula *et al.*, 2009). In the beginning of this year, Westrell and co-workers reported their investigation of several NoV outbreaks linked to oyster consumption, using RT-PCR. The outbreaks occurred simultaneously in the United Kingdom, Norway, France, Sweden and Denmark, representing 334 cases in 65 clusters and NoV was found in samples from 25 of the clusters (Westrell *et al.*, 2010).

Recently, Kostela and co-workers described a long reverse transcription polymerase chain reaction (LRP) using broadly reactive primers (Vennema, de Bruin, & Koopmans, 2002) for amplification of large noroviral RNA sequences in clinical specimens (Kostela *et al.*, 2008). Over the past two decades, the development of molecular diagnostic methods, such as RT-PCR has significantly improved the understanding of NoVs (Patel *et al.*, 2009), and they can be valuable diagnostic tools in the evaluation of outbreak.

Enterovirus

Although, cell culture based diagnostic techniques are reliable and traditionally used for detection of enteroviruses. More and more molecular detection methods such as PCR have been developed during the last decade. Since, cell culture assays are expensive and time-consuming, and molecular techniques offer more sensitive, highly specific and rapid test results for enteroviruses (Hymas, Aldous, Taggart, Stevenson, & Hillyard, 2008; Oberste, Penaranda, Rogers, Henderson, & Nix, 2010). Nowadays, real-time RT-PCR is frequently used for detection of enterovirus in clinical samples, and in some extent also used for detection of enteroviruses in environmental samples (Schwab *et al.*, 1995). A majority of the real-time RT-PCR publish is targeting the highly conserved 5' untranslated region identified by Rotbart *et al.* in 1990 (Rotbart, 1990).

Recently, Rutjes and co-workers reported a comparison evaluation of enterovirus detection by an in-house RT-PCR with a novel commercially available real-time nucleic acid sequence-based amplification (NASBA) assay. Samples of ten liters of raw and treated sewage water and large volumes of river water were analyzed by both methods. They demonstrated that the commercial real-time NASBA assay was somewhat less sensitive than their in-house developed RT-PCR. However, the authors conclude that due to the enormous decrease in turnaround time, the commercial method may be an attractive alternative to RT-PCR

(Rutjes, Italiaander, van den Berg, Lodder, & de Roda Husman, 2005).

Rotavirus

Antigen capture enzyme-linked immunosorbent assay (ELISA), latex agglutination, and reverse transcription-PCR (RT-PCR) electron microscopy are techniques that are relatively efficient to use for detection of rotaviruses. Because of the large quantities of rotavirus present in stool samples all of the methods are suitable for detection of rotavirus in clinical samples. However, PCR has become the best alternative for detection of rotavirus present at low concentrations in environmental samples. A variety of sensitive conventional or RT-PCR methods have been developed based on primers specific for several different rotavirus genes (Kang *et al.*, 2004).

Several studies have demonstrated the use of RT-PCR for detection of the presence of rotaviruses in drinking water-, environmental- and food samples (Kittigul *et al.*, 2008; Meleg *et al.*, 2008).

Meleg and co-workers published development of real-time PCR assay based on broadly reactive primer pair quantitative detection group C rotaviruses in environmental sample. The method was used to analyzed a total of 35 raw and 35 treated sewage samples from four different sewage treatment plants. The result from their study revealed a high detection rate both in case of raw and treated sewage samples, 91% and 57%, respectively (Meleg *et al.*, 2008).

Recently, Kittigul *et al.*, demonstrated development of an RT-nested-PCR method for the detection of rotaviruses in naturally contaminated oyster samples. They, used the method to analyze 120 oyster samples collected from local markets and oyster farms, their study revealed a detection rate of 3.33% (4/120) (Kittigul *et al.*, 2008).

Establishment of microbiological criterias for food borne viruses

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 (food safety 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 risk of food borne viruses has not been established.

Data now exist for NoV in bivalve molluscs to enable the risk managers to establish criteria while considering the impact of a given criteria on the exposure of the consumer. However, sufficient data does not exist yet for HAV.

Example noroviruses

The establishment of microbiological criteria for virus contamination in food will help in the prevention of food borne disease. As an example, microbiological criteria for noroviruses in shellfish could be used. It should now be possible to establish microbiological criteria for noroviruses

in bivalve molluscs shellfish requiring absence in a sample from a food batch. As more knowledge becomes available it can be foreseen that other criteria for noroviruses, HAV, HEV can be established in the years to come to inform risk management decisions.

Another possibility would be testing of food handlers after they have been sick for noroviruses, provided a cheap and reliable testing method is available.

A third possibility would be to have process hygiene criteria for the use of water for irrigation of leafy vegetables intended for direct consumption, but the knowledge of amount of viruses is so far limited, and methods for analyzing larger volumes of water for NoV would then be needed.

Breaking the transmission of viruses along the food chain

One issue would be to introduce handwashing routines when processing foods. Food handlers with clinical symptoms of gastroenteritis indicative of viral origin should be removed from food handling areas and not be allowed to re-enter before after 48 h past the cessation of clinical symptoms or if better diagnostics becomes available 48 h since cessation of virus shedding and clinical symptoms.

In the case that some workers have clinical symptoms their will be need for a general increase in biosecurity of the food processing and handling for all staff as the likelihood person shedding the viruses without having clinical symptoms is increased.

Moreover, the cleaning of surfaces in a food business must have regard that viruses can survive for several weeks. Thus cleaning and disinfection procedures must take this into account.

Concluding remarks

Many studies have been conducted to investigate the presence of virus in the environment. However, much more research is needed, especially on the development of quantitative and qualitative analysis of viruses in water and environmental samples (e.g. food samples). The problem is that virus levels are often so low that they are difficult to detect, yet high enough to pose a health risk.

A reasonable conclusion appears to be that control of food borne viruses cannot be done through solely by test and removal of contaminated foodstuffs, but rather by process control validated and supported by testing for food borne viruses.

Virus in the environment often originates from waste of infected people or animals, which can contaminate both fresh and sea water. Sewage or runoff from agricultural land, contaminated by application of organic waste or manure, can contaminate water, and this water will present a higher risk for contamination if used as irrigation or processing water of foods, or being close to aquaculture sites such as oyster farms. Since viruses do not multiply outside a host, the transmission of enteric viral pathogens from food is an integral part of the cycle of disease. They can

persist in an infectious state until contact with a new host, and they have proved capable of prolonged survival in different environments such as water and food products.

Extensive extraction and concentration procedures are needed before the virus can be detected in food and environment. Various pretreatment techniques have been developed for that purpose; such as filtration, flocculation, and centrifugation. These have been applied in both the European and national projects for the pretreatment of sample before nucleic acid extraction and delivered to final detection system.

In order to choose the most relevant diagnostic assay for detection of virus contamination in food; answering a number some key questions is helpful.

We suggest that the following hierarchy for the possible uses of diagnostic tests is applied.

Is the assay for detection of the virus in question suitable for:

- a. Research purpose: detection, identification and characterization of the strains. It is more interesting what you find than what you might miss.
- b. Outbreak investigations for examining foodstuff linked epidemiologically to human cases. Would a positive finding of virus confirm the epidemiological suspicion regarding source while a negative finding would refute the hypothesis?
- c. Could the detection methods be used for validating the process control (GHP, HACCP) in food production of risk food? In this case, would the absence confirm that the process control is working and a positive finding that there is a health risk with the food production process/chain?
- d. Could the detection methods be used for monitoring surveillance of food sources? Here the costs and resources required to run the procedure and whether there would be many false positives (meaning lots of food are wasted) while if there are false negatives indicating food still unsafe. If you run a monitoring program you could still interpret the results on the population level, but not for the individual sample. A surveillance program could be difficult though.
- e. Could you use the detection method to declare a batch of food safe or unsafe, in this case you must be able to interpret the results on individual batch level. If there would be many false positives (meaning lots of food are wasted) while if there are false negatives indicating food still unsafe.

References

- Anonymous (2000). Report of the study of infectious intestinal disease in England. *Communicable Disease Report Weekly*, 10, 457.
- Baert, L., Uyttendaele, M., Van Coillie, E., & Debevere, J. (2008). 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.
- Baker, K., Morris, J., McCarthy, N., Saldana, L., Lowther, J., Collinson, A., et al. (2010). An outbreak of norovirus infection linked to oyster consumption at a UK restaurant. February 2010. *Journal of Public Health (Oxford, England)*. 2010 Oct 27.
- Balogh, Z., Egyed, L., Ferenczi, E., Ban, E., Szomor, K. N., Takacs, M., et al. (2011). Experimental infection of goats with Tick-Borne Encephalitis virus and the possibilities to prevent virus transmission by raw goat milk. *Intervirology*.
- Butot, S., Le Guyader, F. S., Krol, J., Putallaz, T., Amoroso, R., & Sanchez, G. (2010). Evaluation of various real-time RT-PCR assays for the detection and quantitation of human norovirus. *Journal of Virological Methods*, 167, 90–94.
- Calder, L., Simmons, G., Thornley, C., Taylor, P., Pritchard, K., Greening, G., et al. (2003). An outbreak of hepatitis A associated with consumption of raw blueberries. *Epidemiology and Infection*, 131, 745–751.
- Castkova, J., & Benes, C. (2009). Increase in hepatitis A cases in the Czech Republic in 2008—an update. *Euro Surveillance*, 14.
- Caul, E. O., & Appleton, H. (1982). The electron microscopical and physical characteristics of small round human fecal viruses: an interim scheme for classification. *Journal of Medical Virology*, 9, 257–265.
- Cencic, A. (2008). *EU-WP6. Programme pathogen combat*. Annual report. <http://www.pathogencombat.com>
- Cencic, A., & Avsic-Zupanc, T. (2009). Tick borne encephalitis virus (TBEV). In: *Pathogen combat for safe food: New trends and tools in food safety*. Copenhagen, Denmark: University of Copenhagen.
- Cisak, E., Wojcik-Fatla, A., Zajac, V., Sroka, J., Buczek, A., & Dutkiewicz, J. (2010). Prevalence of tick-borne encephalitis virus (TBEV) in samples of raw milk taken randomly from cows, goats and sheep in eastern Poland. *Annals of Agricultural and Environmental Medicine*, 17, 283–286.
- Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., et al. (2010). Pig liver sausage as a source of hepatitis E virus transmission to humans. *The Journal of Infectious Diseases*, 202, 825–834.
- David Lees, C. W. T. (2010). International Standardisation of a method for detection of human pathogenic viruses in Molluscan shellfish. *Food and Environmental Virology*, 2, 146–155.
- Dumpis, U., Crook, D., & Oksi, J. (1999). Tick-borne encephalitis. *Clinical Infectious Diseases*, 28, 882–890.
- ECDC (2009). *Expert forum on communicable disease outbreaks on cruise ships*. Meeting Report. http://www.ecdc.europa.eu/en/publications/Publications/1002_MER_Outbreaks_on_cruise_ships_final.pdf
- Emerson, S. U., Arankalle, V. A., & Purcell, R. H. (2005). Thermal stability of hepatitis E virus. *The Journal of Infectious Diseases*, 192, 930–933.
- FAO/WHO (2008). *Food and Agriculture Organization of the United Nations/World Health Organization, Microbiological hazards in fresh leafy vegetables and herbs: Meeting report*. In: *Microbiological risk assessment series, No. 14*. Rome, 151pp.
- Fiore, A. E. (2004). Hepatitis A transmitted by food. *Clinical Infectious Diseases*, 38, 705–715.
- Fong, T. T., & Lipp, E. K. (2005). Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. *Microbiology and Molecular Biology Reviews*, 69, 357–371.
- Glass, R. I., Parashar, U. D., & Estes, M. K. (2009). Norovirus gastroenteritis. *The New England Journal of Medicine*, 361, 1776–1785.
- Green, J., Gallimore, C. I., Norcott, J. P., Lewis, D., & Brown, D. W. G. (1995). Broadly reactive reverse-transcriptase polymerase chain-reaction for the diagnosis of Srsv-associated gastroenteritis. *Journal of Medical Virology*, 47, 392–398.
- Gyarmati, P., Mohammed, N., Norder, H., Blomberg, J., Belak, S., & Widén, F. (2007). Universal detection of hepatitis E virus by two

- real-time PCR assays: TaqMan and Primer-probe energy transfer. *Journal of Virological Methods*, 146, 226–235.
- Hafliker, D., Gilgen, M., Luthy, J., & Hubner, P. (1997). Seminested RT-PCR systems for small round structured viruses and detection of enteric viruses in seafood. *International Journal of Food Microbiology*, 37, 27–36.
- Halliday, M. L., Kang, L. Y., Zhou, T. K., Hu, M. D., Pan, Q. C., Fu, T. Y., et al. (1991). An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *The Journal of Infectious Diseases*, 164, 852–859.
- He, X. Q., Cheng, L., Li, W., Xie, X. M., Ma, M., & Wang, Z. J. (2008). Detection and distribution of rotavirus in municipal sewage treatment plants (STPs) and surface water in Beijing. *Journal of Environmental Science and Health, Part A. Toxic/Hazardous Substances and Environmental Engineering*, 43, 424–429.
- Hee-Yeon Kim, I.-S. K., In-Gyun, H., & GwangPyo, K. (2008). Optimization of methods for detecting norovirus on various fruit. *Journal of Virological Methods*, 153, 104–110.
- Hohne, M., & Schreier, E. (2004). Detection and characterization of norovirus outbreaks in Germany: Application of a one-tube RT-PCR using a fluorogenic real-time detection system. *Journal of Medical Virology*, 72, 312–319.
- Holzmann, H., Aberle, S. W., Stiasny, K., Werner, P., Mischak, A., Zainer, B., et al. (2009). Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. *Emerging Infectious Diseases*, 15, 1671–1673.
- Hymas, W. C., Aldous, W. K., Taggart, E. W., Stevenson, J. B., & Hillyard, D. R. (2008). Description and validation of a novel real-time RT-PCR enterovirus assay. *Clinical Chemistry*, 54, 406–413.
- Jiang, X., Huang, P. W., Zhong, W. M., Farkas, T., Cubitt, D. W., & Matson, D. O. (1999). Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses by RT-PCR. *Journal of Virological Methods*, 83, 145–154.
- John, D. E., & Rose, J. B. (2005). Review of factors affecting microbial survival in groundwater. *Environmental Science & Technology*, 39, 7345–7356.
- Jothikumar, N., Lowther, J. A., Henshilwood, K., Lees, D. N., Hill, V. R., & 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. *Applied and Environmental Microbiology*, 71, 1870–1875.
- Kang, G., Iturriza-Gomara, M., Wheeler, J. G., Crystal, P., Monica, B., Ramani, S., et al. (2004). Quantitation of group A rotavirus by real-time reverse-transcription-polymerase chain reaction: correlation with clinical severity in children in South India. *Journal of Medical Virology*, 73, 118–122.
- Katayama, K., Shirato-Horikoshi, H., Kojima, S., Kageyama, T., Oka, T., Hoshino, F., et al. (2002). Phylogenetic analysis of the complete genome of 18 Norwalk-like viruses. *Virology*, 299, 225–239.
- Kerbo, N., Donchenko, I., Kutsar, K., & Vasilenko, V. (2005). Tickborne encephalitis outbreak in Estonia linked to raw goat milk. May–June 2005. *Euro Surveillance*, 10, E050623 050622.
- Kim, H. Y., Kwak, I. S., Hwang, I. G., & Ko, G. (2008). Optimization of methods for detecting norovirus on various fruit. *Journal of Virological Methods*, 153, 104–110.
- Kittigul, L., Pombubpa, K., Rattanatham, T., Diraphat, P., Utrarachkij, F., Pungchitton, S., et al. (2008). Development of a method for concentrating and detecting rotavirus in oysters. *International Journal of Food Microbiology*, 122, 204–210.
- Kocwa-Haluch, R. (2001). Waterborne enteroviruses as a hazard for human health. *Polish Journal of Environmental Studies, Vol. 10*, 485–487.
- Koopmans, M. (2005). Food-borne norovirus outbreaks: a nuisance or more than that? *Wien Klin Wochenschr*, 117, 789–791.
- Koopmans, M., & Duizer, E. (2004). Foodborne viruses: an emerging problem. *International Journal of Food Microbiology*, 90, 23–41.
- Kostela, J., Ayers, M., Nishikawa, J., McIntyre, L., Petric, M., & Tellier, R. (2008). Amplification by long RT-PCR of near full-length norovirus genomes. *Journal of Virological Methods*, 149, 226–230.
- Le Guyader, F. S., Le Saux, J. C., Ambert-Balay, K., Krol, J., Serais, O., Parnaudeau, S., et al. (2008). Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology*, 46, 4011–4017.
- Le Guyader, F. S., Mittelholzer, C., Haugarreau, L., Hedlund, K. O., Alsterlund, R., Pommepuy, M., & Svensson, L. (2004). Detection of noroviruses in raspberries associated with a gastroenteritis outbreak. *International Journal of Food Microbiology*, 97, 179–186.
- Lowther, J. A., Avant, J. M., Gizynski, K., Rangdale, R. E., & Lees, D. N. (2010). Comparison between quantitative real-time reverse transcription PCR results for norovirus in oysters and self-reported gastroenteric illness in restaurant customers. *Journal of Food Protection*, 73, 305–311.
- Maunula, L., Roivainen, M., Keranen, M., Makela, S., Soderberg, K., Summa, M., et al. (2009). Detection of human norovirus from frozen raspberries in a cluster of gastroenteritis outbreaks. *Euro Surveillance*, 14.
- Mbithi, J. N., Springthorpe, V. S., Boulet, J. R., & Sattar, S. A. (1992). Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *Journal of Clinical Microbiology*, 30, 757–763.
- McCausland, K. A., Bond, W. W., Bradley, D. W., Ebert, J. W., & Maynard, J. E. (1982). Survival of hepatitis A virus in feces after drying and storage for 1 month. *Journal of Clinical Microbiology*, 16, 957–958.
- Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., et al. (1999). Food-related illness and death in the United States. *Emerging Infectious Diseases*, 5, 607–625.
- Meleg, E., Banyai, K., Martella, V., Jiang, B., Kocsis, B., Kisfali, P., et al. (2008). Detection and quantification of group C rotaviruses in communal sewage. *Applied and Environmental Microbiology*, 74, 3394–3399.
- Mormann, S., Dabisch, M., & Becker, B. (2010). Effects of technological processes on the tenacity and inactivation of norovirus genogroup II in experimentally contaminated foods. *Applied and Environmental Microbiology*, 76, 536–545.
- Nakayama, M., Ueda, Y., Kawamoto, H., HanJun, Y., Saito, K., Nishio, O., et al. (1996). Detection and sequencing of Norwalk-like viruses from stool samples in Japan using reverse transcription-polymerase chain reaction amplification. *Microbiology and Immunology*, 40, 317–320.
- Newell, D. G., Koopmans, M., Verhoef, L., Duizer, E., Aidara-Kane, A., Sprong, H., et al. (2010). Food-borne diseases - the challenges of 20 years ago still persist while new ones continue to emerge. *International Journal of Food Microbiology*, 139(Suppl 1), S3–S15.
- Niu, M. T., Polish, L. B., Robertson, B. H., Khanna, B. K., Woodruff, B. A., Shapiro, C. N., et al. (1992). Multistate outbreak of hepatitis A associated with frozen strawberries. *The Journal of Infectious Diseases*, 166, 518–524.
- Oberste, M. S., Penaranda, S., Rogers, S. L., Henderson, E., & Nix, W. A. (2010). Comparative evaluation of Taqman real-time PCR and semi-nested VP1 PCR for detection of enteroviruses in clinical specimens. *Journal of Clinical Virology*, 49, 73–74.
- Okamoto, H. (2011). Hepatitis E virus cell culture models. *Virus Research*.
- Parashar, U. D., Hummelman, E. G., Bresee, J. S., Miller, M. A., & Glass, R. I. (2003). Global illness and deaths caused by rotavirus disease in children. *Emerging Infectious Diseases*, 9, 565–572.
- Patel, M. M., Hall, A. J., Vinje, J., & Parashar, U. D. (2009). Noroviruses: a comprehensive review. *Journal of Clinical Virology*, 44, 1–8.
- Perevoscikovs, J., Lucenko, I., Magone, S., Brila, A., Curikova, J., & Vennema, H. (2009). Community-wide outbreak of hepatitis A in Latvia in 2008-an update. *Euro Surveillance*, 14.

- Purcell, R. H., & Emerson, S. U. (2008). Hepatitis E: an emerging awareness of an old disease. *Journal of Hepatology*, *48*, 494–503.
- Rotbart, H. A. (1990). Enzymatic RNA amplification of the enteroviruses. *Journal of Clinical Microbiology*, *28*, 438–442.
- Rutjes, S. A., Italiaander, R., van den Berg, H. H., Lodder, W. J., & de Roda Husman, A. M. (2005). Isolation and detection of enterovirus RNA from large-volume water samples by using the NucliSens miniMAG system and real-time nucleic acid sequence-based amplification. *Applied and Environmental Microbiology*, *71*, 3734–3740.
- Rutjes, S. A., Lodder, W. J., van Leeuwen, A. D., & de Roda Husman, A. M. (2009). Detection of infectious rotavirus in naturally contaminated source waters for drinking water production. *Journal of Applied Microbiology*, *107*, 97–105.
- Schwab, K. J., De Leon, R., & Sobsey, M. D. (1995). Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, hepatitis A virus, and Norwalk virus by reverse transcription-PCR. *Applied and Environmental Microbiology*, *61*, 531–537.
- SCVMPH (2002). *Health & consumer protection Directorate-General, Directorate C-scientific opinions, C2- management of scientific committees; scientific co-operation and network. Opinion of the scientific committee on veterinary measures relating to public health on Norwalk-like viruses*. adopted on 30–31 January 2002.
- Steinmann, J. (1981). Detection of rotavirus in sewage. *Applied and Environmental Microbiology*, *41*, 1043–1045.
- Sumilo, D., Bormane, A., Asokliene, L., Vasilenko, V., Golovljova, I., Avsic-Zupanc, T., et al. (2008). Socio-economic factors in the differential upsurge of tick-borne encephalitis in Central and Eastern Europe. *Reviews in Medical Virology*, *18*, 81–95.
- Tamada, Y., Yano, K., Yatsuhashi, H., Inoue, O., Mawatari, F., & Ishibashi, H. (2004). Consumption of wild boar linked to cases of hepatitis E. *Journal of Hepatology*, *40*, 869–870.
- Tanaka, T., Takahashi, M., Takahashi, H., Ichiyama, K., Hoshino, Y., Nagashima, S., et al. (2009). Development and characterization of a genotype 4 hepatitis E virus cell culture system using a HE-JF5/15F strain recovered from a fulminant hepatitis patient. *Journal of Clinical Microbiology*, *47*, 1906–1910.
- Tei, S., Kitajima, N., Ohara, S., Inoue, Y., Miki, M., Yamatani, T., et al. (2004). Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. *Journal of Medical Virology*, *74*, 67–70.
- Vennema, H., de Bruin, E., & Koopmans, M. (2002). Rational optimization of generic primers used for Norwalk-like virus detection by reverse transcriptase polymerase chain reaction. *Journal of Clinical Virology*, *25*, 233–235.
- Vinje, J., & Koopmans, P. G. (1996). Molecular detection and epidemiology of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. *Journal of Infectious Diseases*, *174*, 610–615.
- Westrell, T., Dusch, V., Ethelberg, S., Harris, J., Hjertqvist, M., Jourdan-da Silva, N., et al. (2010). Norovirus outbreaks linked to oyster consumption in the United Kingdom, Norway, France, Sweden and Denmark, 2010. *Euro Surveillance*, *15*.
- WHO (2004). *The global burden of disease: 2004 update*. http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf
- WHO (2007). *Food safety and foodborne illness*. Fact sheet N°237. <http://www.who.int/mediacentre/factsheets/fs237/en/>
- WHO (2009). *Initiative for vaccine research (IVR)*.
- Widén, F. Workshop (2009). Food borne virus infections. In: *Pathogen combat for safe food: New trends and tools in food safety*. Copenhagen, Denmark: University of Copenhagen.
- Zhai, L., Dai, X., & Meng, J. (2006). Hepatitis E virus genotyping based on full-length genome and partial genomic regions. *Virus Research*, *120*, 57–69.