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Report Series

Foodborne Viruses: An Emerging Problem

Report

**Prepared under the responsibility of the
ILSI Europe Emerging Pathogen Task Force**

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FOODBORNE VIRUSES: AN EMERGING PROBLEM

By Marion Koopmans and Erwin Duizer

REPORT

PREPARED UNDER THE RESPONSIBILITY OF THE ILSI EUROPE EMERGING PATHOGEN TASK FORCE

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EXECUTIVE SUMMARY

Several groups of viruses may infect persons after ingestion and then are shed via stool. Of these, the Norwalk-like caliciviruses (NLV) and hepatitis A virus (HAV) are currently recognised as the most important human foodborne pathogens with regard to the number of outbreaks and people affected in the Western world.

NLV and HAV are highly infectious and may lead to widespread outbreaks. The clinical manifestation of NLV infection, however, is relatively mild. Asymptomatic infections are common and may contribute to the spread of the infection. Introduction of NLV in a community or population (a seeding event) may be followed by additional spread because of the highly infectious nature of NLV, resulting in a great number of secondary infections (50% of contacts).

Hepatitis A is an increasing problem because of the decrease in immunity of populations in countries with high standards of hygiene.

Molecular-based methods can detect viruses in shellfish but are not yet available for other foods. The applicability of the methods currently available for monitoring foods for viral contamination is unknown.

No consistent correlation has been found between the presence of indicator microorganisms (i.e. bacteriophages, *E. coli*) and viruses.

NLV and HAV are highly infectious and exhibit variable levels of resistance to heat and disinfection agents. However, they are both inactivated at 100°C.

No validated model virus or model system is available for studies of inactivation of NLV, although investigations could make use of structurally similar viruses (i.e. canine and feline caliciviruses).

In the absence of a model virus or model system, food safety guidelines need to be based on studies that have been performed with the most resistant enteric RNA viruses (i.e. HAV, for which a model system does exist) and also with bacteriophages (for water).

Most documented foodborne viral outbreaks can be traced to food that has been manually handled by an infected foodhandler, rather than to industrially processed foods. The viral contamination of food can occur anywhere in the process from farm to fork, but most foodborne viral infections can be traced back to infected persons who handle food that is not heated or otherwise treated afterwards. Therefore, emphasis should be on stringent personal hygiene during preparation.

If viruses are present in food preprocessing, residual viral infectivity may be present after some industrial processes. Therefore, it is key that sufficient attention be given to good agriculture practice (GAP) and good manufacturing practice (GMP) to avoid introduction of viruses onto the raw material and into the food-manufacturing environment, and to HACCP to assure adequate management of (control over) viruses present during the manufacturing process.

If viruses are present in foods after processing, they remain infectious in most circumstances and in most foods for several days or weeks, especially if kept cooled (at 4°C). Therefore, emphasis should be on stringent personal hygiene during preparation.

For the control of foodborne viral infections, it is necessary to:

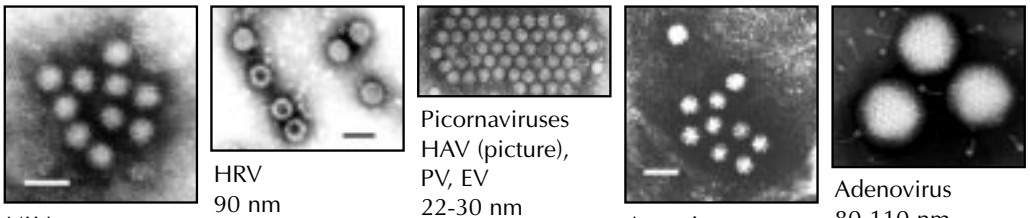
- Heighten awareness about the presence and spread of these viruses by foodhandlers;
- Optimise and standardise methods for the detection of foodborne viruses;
- Develop laboratory-based surveillance to detect large, common-source outbreaks at an early stage; and
- Emphasise consideration of viruses in setting up food safety quality control and management systems (GHP, GMP, HACCP).

INTRODUCTION

Viruses are very small microorganisms, ranging in size from 15–400 nanometers (examples, see Figure 1). Viruses cause a wide range of diseases in plants, animals and humans. These infections do not occur at random: each group of viruses has its own typical host range and cell preference (called tropism). Viruses can be transmitted in different ways, for example by droplets generated when an infected person coughs, by contamination with stool samples from a person infected with an intestinal virus, by sexual intercourse, by contact with blood from infected persons with bloodborne viruses, by contact with infected animals with zoonotic viruses, or by vectors, such as mosquitoes or ticks for arthropod-borne (arbo-) viruses. Clearly the most relevant in foodborne infections are those viruses that infect the cells lining the intestinal tract and are dispersed by shedding into the stool or through emesis (Table 1). Some general features of foodborne viral infections and important differences from foodborne bacterial infections are:

- Only a few particles are needed to produce illness;
- High numbers of viral particles are shed in the stools from infected persons (up to 10^{11} particles per gram stool reported for rotavirus);
- Viruses need specific living cells in order to replicate and therefore cannot do so in food or water; and
- Foodborne viruses typically are quite stable outside the host and are acid resistant.

Figure 1
Electron micrograph and some structural properties of enteric viruses that are commonly (NLV, HAV) or occasionally (other viruses) associated with foodborne or waterborne transmission.
(NLV = Norwalk-like viruses, HAV = hepatitis A viruses, PV = poliovirus, EV = enterovirus, HRV = human rotavirus; ss = single-stranded, ds = double-stranded)



NLV
30-38 nm
ssRNA
no envelope*

HRV
90 nm
dsRNA
no envelope*

Picornaviruses
HAV (picture),
PV, EV
22-30 nm
ssRNA
no envelope**

Astrovirus
28-30 nm
ssRNA
no envelope*

Adenovirus
80-110 nm
dsDNA
no envelope***

* *Photocredit: © F.P. Williams U.S. EPA, with kind permission*
** *Taken from Locarnini et al., 1974, with kind permission*
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Conceivably, current food hygiene guidelines, most of which have been optimised for the prevention of bacterial infections, may be only partially (if at all) effective against viruses. A complicating factor is that most common foodborne viruses grow poorly or not at all in cell culture, so that studies of inactivation of these pathogens are not possible. For this overview, we have reviewed currently available information on foodborne viruses and tried to give an estimate of viral inactivation by looking for parallels in structurally similar viruses that can be grown in cell culture systems in the laboratory.

Table 1
Likelihood of food- or waterborne transmission of enterically transmittable viruses, according to the type of illness associated with infection

	Illness		
Likelihood of food- or waterborne transmission	Gastroenteritis	Hepatitis	Other
Common	Norwalk-like calicivirus	Hepatitis A virus	
Occasionally	Enteric adenovirus (types 40/41) Rotavirus (group A–C) Sapporo-like calicivirus Astrovirus Coronavirus Aichivirus	Hepatitis E virus (waterborne)	Enterovirus*

** Enteroviruses (e.g. poliovirus) are associated with a range of symptoms, including neurological symptoms.*

WHICH VIRUSES ARE INVOLVED?

Numerous viruses can be found in the human gut, but only a few are commonly recognised as important foodborne pathogens. These can be classified into three main groups, according to the type of illness they produce (Table 1):

- Viruses that cause gastroenteritis;
- Enterically transmitted hepatitis viruses; and
- A third group of viruses that replicate in the human intestine but cause illness after they migrate to other organs, such as the central nervous system or the liver.

Foodborne illness has been documented for most of these viruses, but recent studies show that the NLV and HAV are by far the most common cause of illness by this mode of transmission (Cliver, 1997). Some large foodborne outbreaks have occurred with group B and C rotaviruses, and waterborne outbreaks have occurred with hepatitis E virus.

EPIDEMIOLOGY

Recent studies have shown that NLV is the single most common cause of gastroenteritis in people of all age groups and is as common as rotavirus in patients who consult their general practitioners for gastroenteritis (Wheeler *et al*., 1999; Koopmans *et al*., 2000). The incidence is highest in young children, but illness also occurs regularly in adults. Asymptomatic infections are common. In addition, the majority of outbreaks of gastroenteritis in institutions such as nursing homes and hospitals is caused by NLV (Codex Alimentarius, 1999). Although it is not known what proportion of infections can be attributed to the consumption of contaminated food, several reports have shown that foodborne NLV infections are common. Large, even international foodborne outbreaks of NLV have been described (Berg *et al*., 2000). Data from seroprevalence studies suggest that NLV infections are found worldwide.

For HAV the picture is different. The incidence of HAV infection varies considerably among and within countries (Mast and Alter, 1993). In much of the developing world, where HAV infection is endemic, the majority of persons are infected in early childhood and virtually all adults are immune. In these areas, HAV transmission is primarily from person to person. Outbreaks are rare, because most infections occur among young children who generally remain asymptomatic. In the developed countries, however, HAV infections become less common as a result of increased standards of living. Very few persons are infected in early childhood, and the majority of adults remain susceptible to infection by HAV. Because virus shedding starts 10–14 days before the onset of symptoms, there is a clear window for spreading the virus. As a result, the risk of (large) outbreaks of HAV increases in these regions.

In addition, adults are more likely to develop symptoms upon infection, causing enhanced recognition of outbreaks. Indeed, foodborne outbreaks have been reported in most parts of the world and can be large. For example in Shanghai, China, in 1988, 250,000 people had HAV after consumption of contaminated clams (Halliday *et al*., 1991). Detection of sporadic cases or small clusters of foodborne hepatitis A is problematic, because the incubation period can be long. As a result, a possible association with food consumed weeks ago can rarely be investigated at the time of onset of illness. For NLV and HAV, waterborne outbreaks are unusual but have been reported.

ARE FOODBORNE VIRUSES ZOONOTIC?

For most enteric viruses, host range variants have been found in different animal species. So far, however, the majority appear to be quite host specific. Recently, NLV was found in a large proportion of calf herds and in some pigs (Poel *et al*., 2000). The strains in animals were genetically distinct from any of the viruses found in people. No calf-to-human or pig-to-human transmission has been documented so far. The animal viruses, however, are quite similar to the human NLV and continue to change as all RNA viruses do. This implies that zoonotic transmission might occur if the right circumstances arise.

Similarly, hepatitis E virus (HEV) variants were found in pigs, and, in this case, almost identical viruses were found in some humans (Meng *et al*., 1997). This was taken as the first evidence of zoonotic transmission of HEV. The pig viruses appear to be quite common, even in countries where HEV is rarely diagnosed in humans. This suggests that the risk of zoonotic transmission is rather low and currently of no practical consequence for food handling procedures. Again, however, given the genetic flexibility of RNA viruses, these viruses should be monitored closely for changes in behaviour.

DETECTION AND TYPING

Infection with gastroenteritis viruses is usually diagnosed by the detection of the pathogen in stool samples from sick people, rather than by measuring the antibody response in serum (Tables 2, 3). Historically, viruses were diagnosed by scanning a stool suspension under an electron microscope (EM) (Atmar and Estes, 2001). This assay still remains the gold standard for virus diagnosis but is rather insensitive and labour-intensive (Table 2). Routine ELISA assays are available for detection of group A rotaviruses, adenoviruses, and astroviruses, as well as for some of the NLV (Tables 2, 3). For nongroup A rotaviruses, Sapporo-like viruses (SLV), and the remaining NLV, the diagnosis can be made by detection of viral nucleic acid using reverse transcriptase-polymerase chain reaction (RT-PCR) assays. A problem with NLV is the variability of the viral genome, making it difficult to develop a single generic detection test. For the hepatitis viruses, detection of specific IgM antibodies is diagnostic of recent infection. In addition, viruses can be detected in stool and in serum by RT-PCR, but this is not done routinely. There are great differences in the detection limit of the different assays, ranging from a few particles (cell culture and RT-PCR) to a million particles per gram as minimum amounts necessary for a positive test (Table 2). This has direct consequences for the interpretation of results. A person with a positive EM test sheds a great number of viral particles, whereas a person with a positive RT-PCR may shed few particles. No clear guidelines are yet available on interpretation of these different test results, and little has been done to standardise tests. Complicating factors are that people who are ill do not necessarily shed more viruses than those who have no symptoms and that the maximum levels of shedding appear to be different for different viruses. Thus, at present, the diagnosis of viral infection is qualitative (yes/no) and does not provide additional information that may help in deciding whether the person presents an important risk factor for the food chain. On the other hand, because the minimum dose required for infection is very low for these viruses, any infected person may spread the disease.

Table 2
Properties of tests that are used to measure the presence of virus or viral infection

Principle of assay	Example	Infectivity test	Detection limit (particles per gram)
Visualisation of particles	EM	No	$10^{5–6}$
Detection of viral protein	ELISA, latex tests	No	10^{5}
Detection of genome	Probe hybridisation	No	10^{4}
Detection of genome	RT-PCR	No	$10^{1–3}$
Screen for effect on living cells	Cell culture isolation (where feasible)	Yes	$10^{0–1}$
Measurement of exposure	Antibody assays	Yes	Window of detection varies by type of antibody. IgM indicates recent infection.

Virus detection in food or water has been problematic, even after the introduction of RT-PCR. Because the most important foodborne viruses do not grow (readily) in cell culture, they must be detected directly in food extracts, with all the problems of standardisation, inhibition of enzymes used in the RT-PCR, false–positive tests, etc. (Lees, 2000; Atmar *et al*., 2001). Because contamination is often caused by foodhandlers, the level of contamination with virus may vary greatly within a product. The combination of variable virus counts and the lack of a culture system are the main reasons for which virtually no information is available on the variability of test results from sampling or what would be considered representative samples for monitoring purposes. Furthermore, (molecular) diagnostic methods for food or water are not routinely available in food microbiology laboratories (Table 3). Most successful research has focussed on shellfish, but even with published standard protocols, little is known about the performance of such standards in "the field" (e.g. if a batch of oysters does contain some contaminated ones, how likely is it that a virus test will give the right answer?). Therefore, these methods currently cannot be used reliably for quality control and assurance.

Table 3
Detection and typing methods for foodborne viral infections

Virus	**Detection in:**					
	Clinical samples		**Food**		**Water**	
	Methods	**Category***	**Methods**	**Category**	**Methods**	**Category**
Calicivirus 1. NLV 2. SLV	1. Stool, genome detection, EM** 2. Stool, genome detection, EM	1. S-R 2. S-R	1. Genome detection 2. Genome detection	1. S-E 2. S-E	1. Genome detection 2. Genome detection	1. S-R 2. S-E
Hepatitis A virus	serum, antibody detection	R	Genome detection, culture	S-E/R	Genome detection, culture	S-E/R
Rotaviruses 1. Group A 2. Non-group A	1. Stool antigen detection 2. Stool antigen detection, EM	1. R 2. S-E/R	1. Culture, genome detection	1. S-E	1. Culture, genome detection	1. S-E
Adenoviruses	Stool, antigen detection	R	Genome detection	S-E	Genome detection	S-E
Astroviruses	Stool, antigen detection	S-R	Genome detection, culture	S-E	Culture, genome detection	S-E
Enteroviruses	Stool culture	R	Culture	S-E	Culture, genome detection	S-R
Hepatitis E virus	Serum, antibody detection	R	Genome detection	NA	Genome detection	S-E

* *Category of laboratory: R = routine; S-R is routinely available in specialised laboratories; S-E = experimentally available in specialised laboratories; S-E/R= routinely available in some of the specialised laboratories, experimentally available in more.*

** *EM = particle detection by electron microscope.*

USE OF MOLECULAR EPIDEMIOLOGY IN VIRUS TRACING, PROS AND CONS

For all enteric viruses, strains can be divided into subtypes by analysis of the genome. By doing so, common source outbreaks have been diagnosed, even in cases in which links between different outbreaks had not been suspected on the basis of epidemiological investigation (Berg *et al*., 2000). Conversely, molecular strain typing has also been used to disprove links between cases and a suspected source (Marshall *et al*., 2001). At present, a European foodborne virus network, including various public health institutes, uses information on strain typing to trace NLV and HAV outbreaks. The participating groups have agreed to exchange epidemiological and virological information through a central database to identify international common source outbreaks as early as possible (QLK1-1999-00594; for information: marion.koopmans@rivm.nl).

MONITORING FOR THE PRESENCE/ABSENCE OF VIRUSES: THE PROBLEM OF INFECTIVITY

A problem in drafting recommendations for virus control and prevention is that some enteric viruses grow poorly (HAV) or not at all (NLV) in cultured cells (Atmar and Estes, 2001). In addition, no simple animal models are available for experimental studies of virus inactivation. Thus, detection methods currently rely on genome detection by molecular detection techniques such as RT-PCR. A positive signal indicates an intact segment of viral genomic RNA. This does not provide information on virus infectivity. Completely inactivated particles that pose no threat to public health may still contain intact RNA, thus resulting in a positive virus assay. The RNA will eventually be degraded, but it is unknown how long this will take in different environments. In shellfish, inactivation of the virus was followed by rapid degradation (<1 min.) of viral RNA (Slomka and Appleton, 1998). In seawater, however, RNA persisted for days after inactivation of the virus.

In the absence of a culture system for NLV, a common-sense approach is to review information on structurally similar viruses and use those as models for the noncultivatable pathogens. For HAV, a cell-culture-adapted variant has been used, for example in studies addressing heat resistance in different food items (Bidawid *et al*., 2000a). For NLV, structurally similar viruses are the enteroviruses, HAV, and astroviruses. These are all viruses with a single-stranded RNA genome and are approximately 7 kb long, approximately 30 nanometers in size, and similar in capsid structure (no envelope) (Figure 1). Slomka and Appleton (1998) recommended the use of an animal calicivirus (FeCV) for inactivation studies and found that FeCV was clearly less stable than HAV. Because most infectivity and inactivation data are available for the enteroviruses and HAV, we have used these data for our risk estimates (Table 4). It should be clear, however, that these remain estimates and will have to be evaluated carefully.

Table 4
*Food processes, virus inactivation factors, and resulting risk of the product if viruses are present before processing**

Process	Example of food product	Virus inactivation (log10)	Risk of infection of consumer if viruses are present before processing***	Likelihood of presence before processing***	Remarks
Thermal treatments					
Boiling at 100°C	Any liquid food (e.g. milk) or solid food boiled in water	HAV and PV > 4 (Hollinger *et al.*, 1996)	Negligible	Unlikely	Likelihood of presence depending on food; kinetic data lacking
60°C, 30 min (liquids or solid foods)		HAV < 2 (Hollinger *et al.*, 1996) or HAV > 4 (Croci *et al.*, 1999 ; Millard *et al.*, 1987) PV < 2 (Nissen *et al.*, 1996) NLV: incomplete inactivation (Dolin *et al.*, 1972)	Medium		Inactivation in solid foods lower than in liquids; dependent on fat and protein content
Pasteurisation of solid foods (70°C or equivalent, 2 min)	Paté and other cooked meats	HAV < 2 (Millard *et al.*, 1987) FeCV > 3 (Doultry *et al.*, 1999)	Medium	Unlikely	Inactivation dependent on fat and protein content
Pasteurisation of liquids and immediate packing (e.g. HTST 71.7°C for 15 sec)	Milk, ice cream	HAV < 2 (Bidawid *et al.*, 2000a)	Medium	Unlikely	Inactivation dependent on fat and protein content
UHT & aseptic filling (> 120°C)	Long-life milk, other dairy products		Negligible	Unlikely	
Other physical/chemical/ biological processes					
Drying (spray and freeze drying)	Dried milk, instant dried soups, dessert mixes, chocolate	HAV, FeCV < 1 (Doultry *et al.*, 1999; Mbithi *et al.*, 1991)	High	Unlikely**	No information on commercial drying
Freezing	Ice-cream, frozen desserts (containing fruit)	HAV, PV, FeCV < 1 (Hollinger *et al.*, 1996)	High	Possible	
Fermentation	Cheese, yoghurt	No information		Unlikely	Microbial inactivation of viruses is found for sludge (Ward *et al.*, 1982)
Acidification	Fruit juices, still fruit drinks	NLV: pH 2.7, 3h incomplete (Dolin *et al.*, 1972) HAV: pH 1, 5h incomplete (Hollinger *et al.*, 1996)	Medium	Possible	No quantitative data on inactivation

(continued on next page)

Process	Example of food product	Virus inactivation (log10)	Risk of infection of consumer if viruses are present before processing***	Likelihood of presence before processing***	Remarks
Homogenisation		Incomplete	High		Likelihood of presence depending on type of product
Depuration of oysters and mussels		NLV incomplete (Grohmann *et al.*, 1981)	High	Likely	
High hydrostatic pressure (600 MPa, 1h)		PV < 1 (Wilkinson *et al.*, 2001)	High		Likelihood of presence depending on type of product
Virus inactivation in water				Possible (drinking water) Likely (surface water)	
Chlorination (0.5 mg free chlorine/liter, 1 min)		HAV > 3, HAV < 2 HRV < 2, PV > 3 (Abad *et al.*, 1994; Sobsey, 1989)	Variable		Risk is low for PV but medium for HRV and HAV
UV radiation (20 mJ/cm^2)		PV 3 or less (Sommer *et al.*, 1989) HRV < 3 (Sobsey, 1989)	Low		
Ozone treatment (0.2 mg/L, 10 min)		HAV >3 PV 2 or less HRV < 1 (Kim *et al.*, 1999; Sobsey, 1989)	Variable		Risk is low for HAV but medium/high for PV and HRV
Cleaning of equipment and surfaces					
Rinsing with (lots of) water		HAV < 2 (Bidawid *et al.*, 2000b)	Medium/low		
Ethanol (70%, 10 min)		HAV < 2 HRV < 3 (Abad *et al.*, 1997)	Medium		
Chlorhexidine digluconate (0.05%, 10 min)		HAV < 1 HRV < 1 (Abad *et al.*, 1997; Kawana *et al.*, 1997)	High		
Sodium hypochlorite (0.125%, 10 min)		HAV < 3 HRV < 3 (Abad *et al.*, 1997; Kawana *et al.*, 1997)	Low		

(continued on next page)

Process	Example of food product	Virus inactivation (log10)	Risk of infection of consumer if viruses are present before processing***	Likelihood of presence before processing***	Remarks
Sodium chlorite (30%, 10 min)		HAV > 3 HRV > 5 (Abad *et al.*, 1997)	Negligible		
Catering					
Washing, rinsing (where water > 1% of food) and the food is eaten without additional cooking	Washed salads Fruits (strawberries)	No substantial removal or inactivation	High	Possible	Any removal of viruses will be by mechanical action only; very difficult to remove any microorganisms from foods by washing alone (Mariam and Cliver, 2000b)
Freezing of drinking water to prepare ice	Ice for drinks or for cold foods	No inactivation	High	Possible	Freezing is an excellent way to preserve viruses; therefore best to assume there will be no inactivation after one freeze/thaw cycle
Chilling of drinking water or use of water from tap without any treatment		No inactivation	High	Possible	Chilling will slow down the inactivation rate of viruses

* *Viruses for which data were used to assemble this table are the (common) foodborne hepatitis A virus (HAV), Norwalk-like viruses (NLV) (and the animal model viruses feline calicivirus [FeCV] and canine calicivirus [CaCV]), human rotavirus (HRV), rhesus rotavirus (RV), and poliovirus (PV). Note: estimates included in this table are based on extrapolation of data from scientific studies and should be regarded as indicative only. Data in this table cannot be used to calculate risks. For precise process calculations or predictions on food manufacturing processes, additional experimental information is needed.*

** *Before spray drying in dried milk processes, a substantial heat step destroys viruses.*

*** *Unlikely = no reports are known in which NLV, HAV, RV, or PV were found on these food items.*
Possible = sporadic contamination with NLV, HAV, RV, or PV has been reported on these food items.
Likely = contamination with NLV, HAV, RV, or PV is reported frequently on these food items.
Negligible risk = product highly unlikely to contain infectious viruses; treatment results in at least 4 $\log_{10}$ inactivation of common foodborne viruses.
Low risk = product unlikely to contain infectious viruses in numbers likely to cause disease in healthy individuals; treatment results in approximately 3 $\log_{10}$ inactivation of common foodborne viruses.
Medium risk = product may contain infectious viruses in numbers that may cause disease; treatment results in approximately 2 $\log_{10}$ inactivation of common foodborne viruses.
High risk = products in which the level of viruses is likely to be high enough to cause disease in healthy individuals; treatment results in less than 1 $\log_{10}$ inactivation of common foodborne viruses.
Variable risk = treatment results in significant differences in inactivation of several common foodborne viruses.

CORRELATION BETWEEN INDICATOR ORGANISMS AND VIRUS PRESENCE

In water quality research, the use of indicators for the presence of human pathogenic viruses has been an area of considerable debate (Lees, 2000). It is clear from numerous outbreaks that the presence of "traditional" bacterial indicators of faecal contamination does not consistently correlate with the presence of pathogenic viruses. Many groups have proposed the use of bacteriophages as indicators. Bacteriophages are viruses that infect and replicate in bacteria. They are present in substantial numbers in human stool samples and, in some respects, are similar to viruses pathogenic to humans. Because of similarities in structure, behaviour, and stability, bacteriophages may be of use in assessing cumulative exposure to human faecal waste. However, care must be taken not to provide a false sense of safety by measuring the presence of bacteriophages only. The observed clear differences in stability of different human pathogenic viruses (described later in this document) that reside in the intestine illustrate that extrapolation of data from one virus to another cannot be relied upon (Slomka and Appleton, 1998). Similarly, the possible use of phages as surrogates in evaluating the antiviral effectiveness of processes needs to be carefully validated (Mariam and Cliver, 2000a).

HOW DO FOODS BECOME CONTAMINATED?

Foods can be contaminated by (Figure 2):

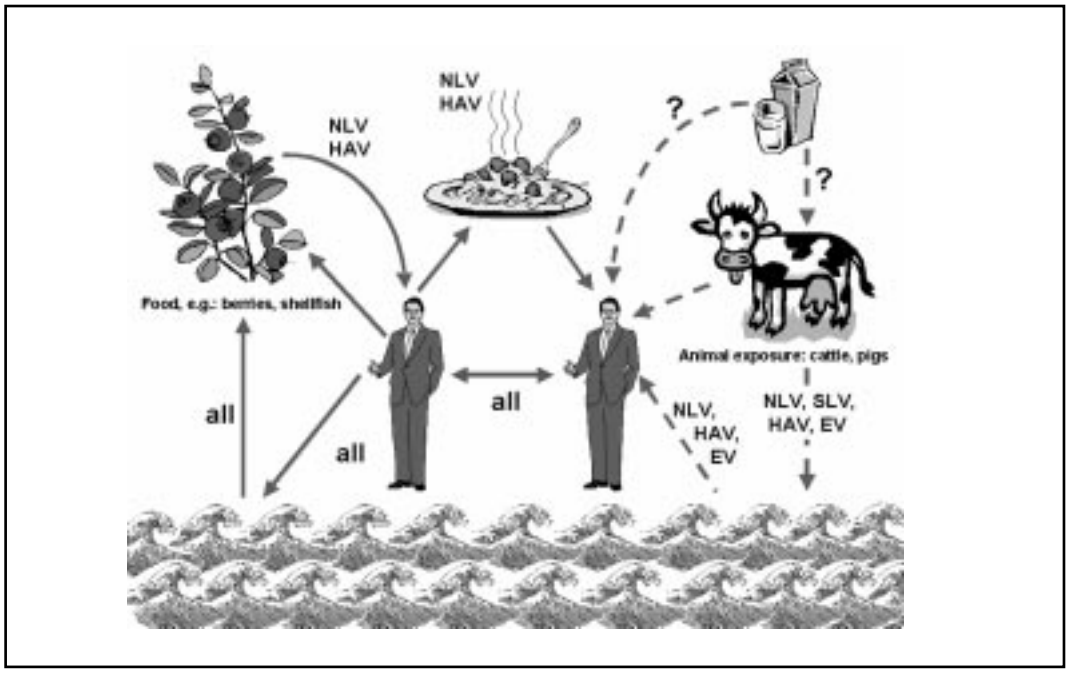
- Contact with (human) faeces or faecally contaminated water;
- Contact with faecally soiled materials (including hands);
- Contact with vomit or water contaminated with vomit;
- Contact with environments in which infected people were present, even if the surface was not directly contaminated with stool or vomit; and
- Aerosols generated by infected people.

There is no proof that animal contact, directly or indirectly (pigs, calves, surface-contaminated meat, meat products, or other products derived from those animals), can be a source of foodborne infection.

Central to the issue are infected foodhandlers. These may be:

- Infected foodhandlers with symptoms. Shedding of virus occurs during the period of illness;
- Infected foodhandlers who have recovered from illness. Shedding of NLV may persist for at least 3 weeks after recovery;
- Infected foodhandlers without symptoms. Asymptomatic infections are common for all foodborne viruses. For example carriers of hepatitis A typically shed high quantities of the virus 10–14 days after infection; in the weeks following this period carriers may or may not develop symptoms; and
- Foodhandlers with contacts with sick people (e.g. people with sick children or relatives).

Figure 2
Modes of transmission of enteric viruses, showing proven (continuous) and suspected (dashed lines) routes of exposure.



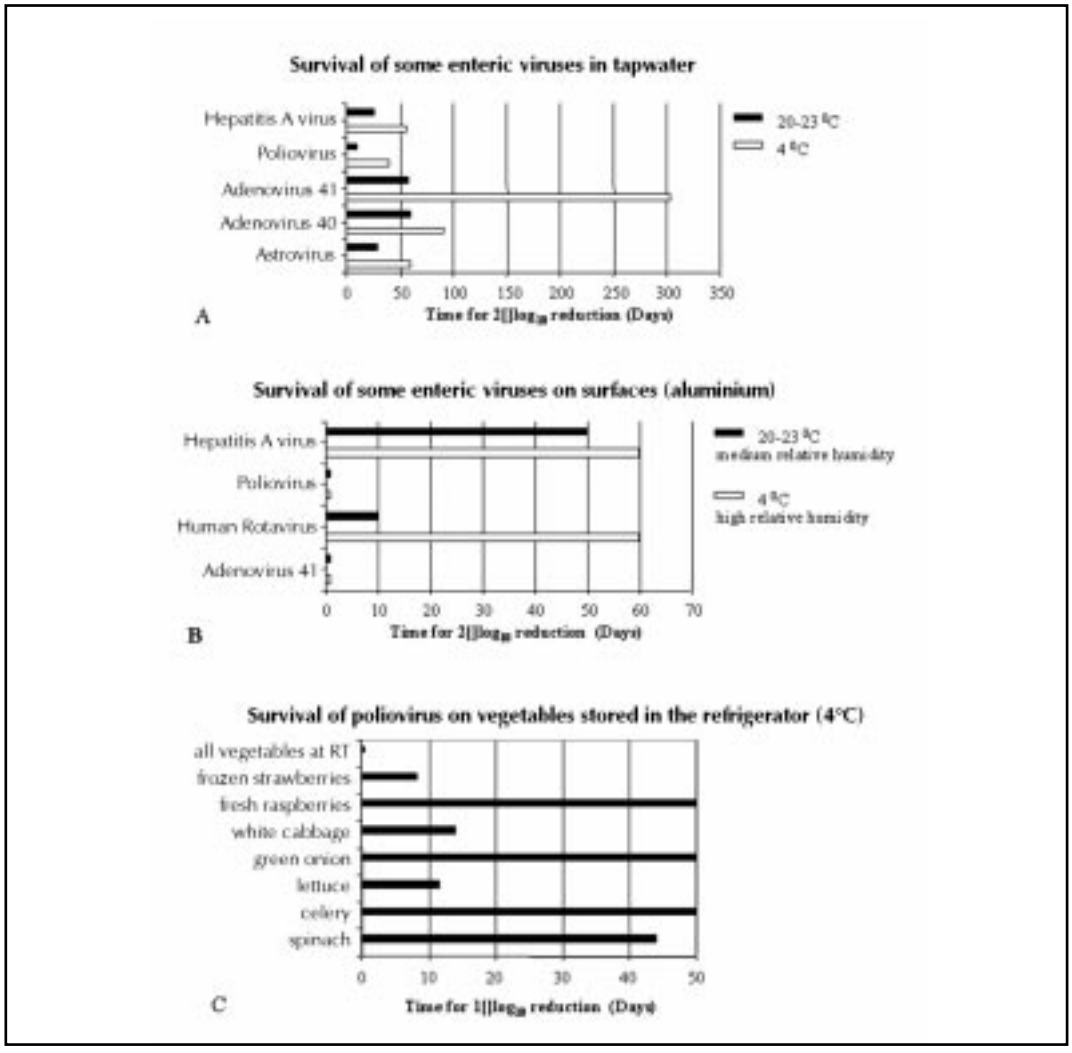
Note that although most outbreaks can be traced to infected foodhandlers at the end of the food chain, they may be anywhere (e.g. seasonal workers picking berries for use in composite foods, people on recreational boats near shellfish harvesting areas, etc.) A large, multistate outbreak of illness associated with oysters was finally traced back to a sick oyster harvester who had vomited and disposed of the waste overboard (Berg *et al.*, 2000).

Outbreaks have been documented in association with a long list of food items (e.g. deli meat, sandwiches, bread rolls, bakery products, berries, ice cubes). Dishes containing fresh (or fresh frozen) fruits and vegetables have been the source of numerous outbreaks of foodborne illness. Filter-feeding shellfish are a particular risk, as they concentrate viruses present in their growing waters, and numerous outbreaks linked to the consumption of shellfish have been reported.

SPREAD AND PERSISTENCE OF FOODBORNE VIRUSES FROM FARM TO FORK

It is clear that foods requiring either intensive manual handling, manual handling under poor hygienic conditions (e.g. in orchards), or close-to-fork and end-product manual handling are the products at highest risk (see www.who.int/fsf/fos982~1.pdf). Information obtained with HAV suggests that approximately 10% of the virus particles can easily be transferred from faecally contaminated fingers to foods and surfaces (Bidawid *et al.*, 2000b).

Figure 3
Virus survival in tapwater (A), aluminium fomites (B), or vegetables (C). Represented are the number of days after which the virus recovery will be less than 1% (A and B) or 10% (C) of the original contamination. (Data from: Enriquez et al., 1995; Kurdziel et al., 2001, Mbithi et al., 1991; and Ward and Irving, 1987)



Another factor determining risk for contamination of foods is the stability of some of the foodborne viruses in the environment. For example, rotaviruses in aerosols (generated while vomiting and thought to play a role in the transmission of those viruses) were found to survive in the air up to 9 days at 20°C (Sattar *et al*., 1984). Viruses also may persist for extended periods (1–60 days for 100-fold reduction in infectivity) on several types of materials commonly found in institutions and domestic environments (e.g. paper, cotton cloth, aluminium, china, glazed tile, latex, and polystyrene; Abad *et al*., 1997) (Figure 3). Adenoviruses were found to survive for up to 35 days on a plastic surface in an environment with low relative humidity (Nauheim, 1990). This relation with humidity varies among viruses. A high relative humidity favours the survival of enteroviruses, whereas a low relative humidity favours survival of HAV and human rotavirus (HRV) (Mbithi *et al*., 1991; Sattar *et al*., 1986; Sattar *et al*., 1988). Furthermore, HAV remained infectious in dried faeces for 30 days when stored at 25°C and 42% relative humidity (Hollinger *et al*., 1996). This stresses the need for virus-specific studies to address virus inactivation. Finally, in artificially contaminated water, viruses may survive for prolonged periods of time, with over 1 year survival of poliovirus and rotavirus in mineral water at 4°C (Biziagos *et al*., 1988). Recent data published by Beuret *et al*. (2000) on traces of NLV RNA found in bottled waters may tend to support this statement. However, as yet, no one has been able to confirm these data.

STABILITY OF FOODBORNE VIRUSES DURING PROCESSING

Viruses, unlike bacteria, are strict intracellular parasites and cannot replicate in food or water. Therefore, viral contamination of food will never increase during processing, transport, or storage, and the contaminated products will look, smell, and taste normal. Moreover, because contamination is often caused by foodhandlers, the level of contamination with virus may vary greatly within a product.

Nonetheless, several recent studies were performed to determine the modes of transfer and inactivation profiles of foodborne viruses. Most food- or water-borne viruses are more resistant to heat, disinfection, and pH changes than are most vegetative bacteria. It is no coincidence that most virus groups implicated in outbreaks are small, nonenveloped particles, rather than large, fragile, enveloped viruses (Figure 1). Numerous studies have addressed the stability of viruses under different circumstances (see Table 4), but little was done to standardise these studies. An overall conclusion is that HAV and HRV are more resistant to inactivation than enteric adenovirus and poliovirus, but it must be noted that significant differences in survival rates were found for different environmental and substrate conditions. Again, these findings stress the need for independent assessment of behaviour for different viruses.

This poses a problem for NLV, which cannot be grown in cell culture and therefore cannot readily be tested under the experimental conditions described previously. It remains to be seen whether other viruses that can be grown in tissue culture may serve as models for the NLV, as has been suggested for FeCV. In the interim, we recommend using the inactivation profiles of the most stable enteric RNA virus to assess the safety of a process. Thus, for most processes relevant in the food industry, HAV may be considered a good indicator virus.

Information obtained with HAV shows that more than 1000 virus particles can easily be transferred from faecally contaminated fingers to foods and surfaces (Bidawid *et al.*, 2000b). Based on this information, an inactivation factor of at least 3 log10 during post-manual-treatment processes would be required. Based on these assumptions, we have tried to estimate the likelihood of survival of the most important foodborne viruses for commonly used food processing methods if foods are contaminated before processing. With the exception of ultrahigh temperature treatment, no methods would completely inactivate more than 3 $\log_{10}$ of virus, and we estimate that with foods contaminated after processing, viruses will remain active to a significant extent and thus pose a possible risk factor (Figure 3, Table 4). Therefore, the emphasis should be strongly on prevention of contamination before or during processing by proper deployment of GHP, GMP, and HACCP. Clearly, the likelihood of virus contamination in primary products will differ for different commodities and is the highest for shellfish and manually handled fruits. For foods contaminated after processing, our estimate is that viruses will remain active in most foods (Kurdziel *et al.*, 2001) (Figure 3, Table 4).

WHAT CAN BE DONE FOR PREVENTION?

It is clear that most problems with foodborne viruses occur from contamination of food products during manual handling in combination with minimal processing of foods afterwards. With viral infections (e.g. NLV) being very common, it is wise to assume that the introduction of viruses into the food chain is a likely event that needs to be prevented by stringent hygienic control. Foodhandlers in contact with people with gastroenteritis (e.g. young children) are at special risk of being contaminated and becoming a source of viruses during food manufacture operations. They must be made aware that specific personal hygiene must be ensured. Increasing the awareness of all foodhandlers about transmission of enteric viruses (including the spread of viruses by vomiting) is needed, with special emphasis on the risk of "silent" transmission by asymptomatically infected persons and via those who continue to shed virus after recovery from illness. At present, insufficient data are available to determine which steps will be critical for all foods in an HACCP system, but it is clear that at least the following points should be addressed:

- Water used in combination with the culturing or preparation of food should be of drinking water quality; and
- Guidelines specifically aimed at the reduction of viral contamination are needed, as it has become clear that current indicators for water and shellfish quality are insufficient as predictors of viral contamination.

Documented outbreaks of foodborne infections could be reported faster using, for example, the European Foodborne Virus Network, the "rapid alert system for food" of the European Union. These networks could operate more effectively if typing information for virus strains were included.

A vaccine is available for hepatitis A, and contacts can be treated with the administration of immunoglobulin within 2 weeks after exposure. The Advisory Committee on Immunization Practices (ACIP, 1996) in the United States has suggested that HAV vaccination should be considered for foodhandlers, although risk assessment will be different for each country given the great differences in seroprevalence of HAV. At present, most countries prefer to stress the use of stringent personal hygiene to prevent infections.

RECOMMENDED AREAS FOR RESEARCH

- More developmental work is required on methods to detect viruses in food. Such methods should be reasonably simple (as few steps as possible), efficient (in terms of recovery of viruses), and reproducible. The crucial (and most difficult to achieve) step is extraction of virus particles from the food matrix. Research should focus on this process.
- A standard method to assess virus survival would allow acquisition of comparative data (e.g. responses of different virus types to the same set of environmental conditions). The features of this method would include similar inoculum size, sample size, sampling time, and statistical analysis. A project to develop such a method and apply it in various environments would provide useful data.
- Efforts to find a cell culture system that will allow propagation of NLV are vital. The above two recommended areas of endeavour will depend upon this to be applicable to NLV.
- Information is needed on virus survival on different food commodities, including thermal resistance.
- Information is needed on duration of shedding and levels of virus shedding in persons with and without symptoms.

CONSIDERATIONS FOR GOVERNMENTS

- The existing surveillance systems for foodborne viruses are incomplete. Basic virus detection and typing methods are not routinely available in many countries. Rapid detection and reporting networks for foodborne viruses need to be implemented in standard surveillance systems. These networks should combine laboratory and epidemiological information. A reporting strategy for international outbreaks should be established.
- The detection and prevention of foodborne viral infections should be organised. Foodborne viral infections are diagnosed with increasing frequency. This illustrates the existence of regular breaks in the microbial safety of food. Although the most common pathogens cause relatively mild, self-limiting illness, their high incidence illustrates the potential for large, international foodborne viral epidemics. This includes the risk of foodborne spread of more dangerous pathogens, such as HEV or enteroviruses that may cause paralytic illness. Person-to-person spread is very high. As a result, an initial point-source outbreak may be amplified significantly.
- Incidents (foodborne outbreaks) should be evaluated carefully by governments, WHO, and NGOs to identify whether changes in the guidelines are needed.

CONSIDERATIONS FOR AGRICULTURE INDUSTRIES

- The emphasis should be on GAP. Primary products and raw materials, especially those of agricultural origin, must be protected from contamination by human, animal, domestic, or agricultural wastes that are known sources of viruses/microorganisms. Examples of such products are fresh berries and salad.
- Foodhandlers, including seasonal workers, need to be educated specifically about microbial safety guidelines and hygiene rules. This includes education about the risk of exposure to viruses through sick children in the household.
- Managers of agricultural businesses involving produce to be eaten raw need to exclude foodhandlers with symptoms consistent with exposure to infectious foodborne diseases until 48 hours after recovery (Cowden *et al*., 1995). Foodhandlers returning to work need to be instructed that substantial numbers of NLV may be shed for weeks after recovery from illness and that they need to follow hygiene rules strictly.
- The microbial safety guidelines for shellfish need to be revised to include viral food safety.
- Primary products must not be produced in areas where water used for irrigation might constitute a health hazard to the consumer through the food.

CONSIDERATIONS FOR FOOD MANUFACTURING INDUSTRIES

- Food safety management systems (HACCP, GHP and GMP), safety guidelines, and best-practice documents need to include considerations on the possible risks that infectious foodborne viruses pose during and after processing. This underlines the importance of adherence to good personal hygiene.
- Primary products must not be produced in areas where water used for irrigation might constitute a health hazard to the consumer through the food.
- Foodhandlers, including seasonal workers, need to be educated specifically about the microbial safety guidelines and hygiene rules. This includes education about the risk of exposure to viruses through sick children in the household.
- Managers of food manufacturing industries should consider excluding foodhandlers with symptoms consistent with exposure to infectious foodborne diseases until 48 hours after recovery (Cowden *et al*., 1995). Foodhandlers returning to work need to be instructed that NLV can be shed for weeks following recovery from illness and should be made aware that stringent personal hygiene must be ensured.
- Microbial food safety guidelines should be revised to include viral food safety (e.g. for codes of practice).
- Incidents should be reported to public health authorities through existing networks.

CONSIDERATIONS FOR THE CATERING AND FOOD SERVICE INDUSTRIES

- Foodhandlers, including seasonal workers, need to be educated specifically about the microbial safety guidelines and hygiene rules. This includes education about the risk of exposure to viruses through sick children in the household.
- Managers of catering and food service industries need to exclude foodhandlers with symptoms consistent with exposure to infectious foodborne diseases until 48 hours after recovery (Cowden *et al*., 1995). Foodhandlers returning to work need to be instructed that substantial numbers of NLV may be shed for weeks after recovery from illness.
- Incidents should be reported to public health authorities through existing networks.

CONSIDERATIONS FOR CONSUMERS

- Consumers and physicians need to be specifically educated about microbial safety guidelines and hygiene rules, including those for viruses.

FACT SHEET

Foodborne infections by Norwalk-like caliciviruses (small round structured viruses, SRSV).

Introduction

Human enteric caliciviruses cause gastroenteritis in humans. The human caliciviruses are assigned to two groups, the genera Norwalk-like virus (NLV) and Sapporo-like virus (SLV). The NLV are also known as "small-round-structured-viruses" (SRSV), and the SLV as "typical caliciviruses". The two virus groups differ epidemiologically. The NLVs cause illness in people of all age groups, whereas the SLV predominantly cause illness in children.

Foodborne transmission of caliciviruses is well known for viruses in the NLV genus. Within this genus is a great diversity of virus types, with genetic differences and differences in the protein composition of the virus particles. To date, 15 distinct genotypes have been recognised, but their number is likely to increase. Infected persons develop immunity, which is short lived and predominantly type specific. As a result, one person can have multiple NLV infections, which in part explains the high incidence of NLV infection.

Clinical symptoms

After a 1–3-day incubation period, infected persons may develop low-grade fever, vomiting, diarrhoea, and headache as prominent symptoms. Symptoms usually subside within 2–3 days, although the course of illness may be protracted in the elderly. Deaths associated with NLV outbreaks have been reported, but a causative relationship remains to be proven. The average attack rate is high (typically 45% or more). The virus is shed via stools and vomit, starting during the incubation period and lasting up to 10 days and possibly longer. NLV infections are highly contagious, resulting in a high rate of transmission to contacts.

Incidence

NLV infections are among the most important causes of gastroenteritis in adults, and often occur as outbreaks that may be foodborne. The spread can be epidemic. In The Netherlands, approximately 80% of outbreaks of gastroenteritis reported to municipal health services are caused by NLVs. More than half of these outbreaks occur in nursing homes, but this may be an overrepresentation resulting from selection bias. In The Netherlands, foodborne outbreaks are also reported through a network of food inspection services. Preliminary results from studies there suggest that NLVs may also cause a significant number of these outbreaks. Based on studies from the UK and the US, it has been estimated that a substantial proportion of foodborne infections may be caused by NLVs (67% estimated for the US by Mead *et al*, 1999). In addition to outbreaks, NLVs also cause numerous sporadic cases of gastroenteritis. Five percent of patients with gastroenteritis who consult a physician have NLV infection, compared with 4% for *Salmonella*. In addition, caliciviruses are by far the most common cause of sporadic gastroenteritis (NLV accounts for 11% of all cases). Monitoring of sewage samples confirmed that high levels of NLVs circulate in the general population.

Epidemiology

It has been established that many different types of NLV cocirculate in the general population, causing sporadic cases and outbreaks. However, occasionally epidemics occur in which the majority of outbreaks are caused by a single genetic type (e.g. in the Netherlands in 1996).

These epidemics may be widespread and even global. The mechanisms behind the emergence of epidemic types are unknown. Hypotheses range from large-scale foodborne transmission of a single strain to spill over from a reservoir, possibly nonhuman. An indication for the latter was a recent study from Japan in which NLVs were found in stool specimens from pigs, using RT-PCR assays based on caliciviruses of humans (Sugieda *et al.*, 1998*). In the 1990s, the reported incidence of NLV increased, probably as a result of improved diagnostic methods and increased awareness.

Risk groups

Outbreaks of NLV gastroenteritis (not only foodborne) are common in institutions such as nursing homes and hospitals. The high attack rate in both residents and personnel at such institutions leads to major logistic problems (understaffing) during outbreaks. In addition, an unknown but probably large number of sporadic cases occur. The risk factors for these infections are currently under investigation in the United Kingdom and the Netherlands.

Routes of transmission

NLVs are transmitted by direct contact or indirectly via contaminated water, food, or from the environment. Many foodborne NLV outbreaks have been described, often caused by infected foodhandlers. The NLV usually are shed in large quantities during the initial stages of the illness, with maximal titres as high as 10^8 virus particles per gram of stool. Although there are some indications for aerogenic transmission of NLV, the importance of this route is still unclear. Infectious viruses can be transmitted not only at the time of illness but also during the incubation period and after recovery, with 30% of cases shedding virus for up to 3 weeks after infection.

In addition to foodborne transmission, waterborne transmission of NLV is common, both directly (e.g. during recreation) or indirectly. NLVs can survive outside the host, are resistant to common disinfectants and extreme pH fluctuations, and are highly infectious. As a result, transmission of virus via fomites is likely.

High-risk foods

Filter-feeding shellfish are notorious as a source of foodborne viral infections, because they actively concentrate viruses from contaminated water. Infectious viruses can be detected for up to 6 weeks without any loss in quality of the shellfish. Depuration, a practise that may reduce bacterial contamination, is not as effective in reducing the viral load of shellfish.

In addition to shellfish, many food items have been associated with NLV outbreaks. In the literature, several other manually handled foods have been implicated (desserts, fruits, vegetables, salads), but the message is that any food that has been handled manually and not heated (sufficiently) afterwards may be a source of infection.

Diagnosis in humans

NLV or SLV infections can be diagnosed by visualisation of virus particles by electron microscopy and with molecular methods (RT-PCR). However, in most countries these methods are not available for routine diagnostics. Stool viruses can be typed by sequence analysis or by reverse-line blotting, and genetic typing may be used to trace common source outbreaks. Using these techniques, outbreaks from geographically distinct regions have been linked.

*Sugieda, M., Nagaoka, H., Kakishima, Y., Ohshita, T., Nakamura, S., Nakajima, S. (1998). Detection of Norwalk-like virus genes in the caecum contents of pigs. *Arch. Virol.* 143:1215–1221.

Virus detection in food and water

Molecular methods have been adapted for the detection of NLVs in food and water. However, because little is known about their sensitivity under field conditions, these techniques are not yet routinely available. Quality control of food and water on the basis of the detection of indicator organisms for faecal contamination has proven to be an unreliable predictor for viral contamination. When NLVs are detected in food, typing assays can be used to establish transmission routes. However, these techniques are not routinely available in most laboratories.

Zoonotic transmission

Some groups of animal caliciviruses have a broad host range, and it is currently a matter of debate whether NLVs can be transmitted between humans and animals. Recently, caliciviruses indistinguishable from NLVs have been found in pigs in Japan and cattle in the United Kingdom, Germany, and the Netherlands. Data from the Netherlands suggest a very high prevalence of NLV in calf herds.

Prevention of foodborne NLV infections

Strict implementation of hygienic rules is currently considered the most important preventive measure. Foodhandlers with gastroenteritis should immediately be removed from the food chain. More problematic are outbreaks linked to asymptomatic, presymptomatic, and postsymptomatic shedders. The kinetics of viral shedding have been studied in only a few infected volunteers and may not reflect real-life situations in which people may have been infected with a low dose of infectious virus. Given the highly infectious nature of NLV and the documented risk of virus transmission to food during the incubation period, it is suggested that guidelines be developed that include the occurrence of gastroenteritis in contacts (e.g. children) of people working at critical points in the food chain. This should be based on data on the kinetics of viral shedding after natural infection.

For prevention of foodborne transmission, it obviously is also essential that food items be not grown or washed in faecally contaminated water. However, the globalisation of the food market has hampered the implementation of control measures to assure safe food, as it is often difficult to exactly trace the food.

Routine monitoring is not yet feasible, first, because there are no good methods, and, second, because end-product testing is not reliable to assure food safety on statistical grounds. Documented outbreaks of foodborne infections could be reported faster using a system such as the "rapid alert system for food" of the European Union. However, this would be much more informative if typing information of virus strains were included.

Disinfection

Norwalk virus (one of the prototypes NLV) is resistant to low pH and heat treatment (30 minutes at 60°C). The virus reportedly is quite resistant to chlorine; the virus remains infectious after 30 minutes in the presence of 0.5–1 mg free chlorine per litre. At higher concentrations (>2 mg/L free chlorine), the virus is inactivated. The effect of other disinfectants on NLV infectivity has hardly been studied, because of the lack of a tissue culture system or animal model.

ABBREVIATIONS AND DEFINITION

ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
FeCV	feline calicivirus
GAP	good agriculture practice
GHP	good hygienic practice
GMP	good manufacturing practice
HACCP	hazard analysis critical control point
HAV	hepatitis A virus
HEV	hepatitis E virus
HRV	human rotavirus
NGO	nongovernmental organisation
NLV	Norwalk-like virus
RT-PCR	reverse transcriptase polymerase chain reaction
SLV	Sapporo-like virus
WHO	World Health Organization

Foodhandler:
A foodhandler is defined as any person who works in an area where food is being prepared, produced, served, or packed, including those who handle immediate wrapping materials, bulk containers, and machines and those responsible for maintaining and cleaning the workplace, implements, machines, and vehicles. On sites handling "high-risk" foods, all personnel who work in food areas should be included. Workers who handle only pre-wrapped, canned, or bottled food are not considered to be foodhandlers.

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