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A REVIEW

Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection

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1. SUMMARY

Enterically infecting viruses are ubiquitous agents, mostly inducing silent infections. Several are however associated with significant diseases in man from diarrhoea and vomiting to hepatitis and meningitis. These viruses are drawn from a variety of virus families and have different structures and genetic material, yet all are suited to this means of transmission: Normally they are shed in high numbers (assisting environmental transit) and exhibit great particle stability (permitting survival both outside the body and on passage through the stomach). Human activities particularly associated with food and water processing and distribution have the capacity to influence the epidemiology of these viruses. This review provides a description of viruses spreading by these means, their significance as pathogens and considers their behavior in these human-assisted processes.

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2. INTRODUCTION

The term virus stems from the Latin *virus* meaning 'poison', and in some ways virus contamination of food resembles toxic contamination more than contamination with other micro-organisms. Viruses are not free living; they are dormant between hosts and have an absolute requirement for living cells in which to replicate. Human viruses require human cells in which to replicate, these are not present in our food and thus such viruses cannot increase in number during storage. The amount of any contaminating viruses should actually decline during storage, and this can be assisted by treatment with chemicals, heat or irradiation. Human viruses do not cause food spoilage and contamination may provide no visible clues to its presence. These features mean that measures to control bacteria will not

necessarily control viruses and could actually preserve them. Food-borne viruses are infectious at very low doses and could be introduced at any point in the food chain. Many are difficult (or currently impossible) to culture and detection is no simple task. Outbreaks and sporadic occurrences of food-borne virus infection continue throughout the world. There are simply too many to mention and there is no complete data set. It seems likely that the documented outbreaks are limited only by our ability to document them.

The cost of these events is likely to be phenomenal to the community; a single food-borne outbreak of hepatitis A virus (HAV) exposed up to 5000 persons in Colorado. In this case the costs for medical treatment of those infected amounted to approx. $50 000 whilst the cost of tracing and controlling this single outbreak cost over half a million US$ (Dalton *et al.* 1996). The burden of infectious intestinal disease (IID) in its broader sense is likewise huge, in the UK the cost per case of norovirus (NoV)-induced gastroenteritis involving a GP visit is estimated at £176 and two persons in every 1000 will make such a visit each year (FSA 2000). The interested reader is referred to several recent reviews (Lees 2000; Seymour and Appleton 2001; Sair *et al.* 2002; Koopmans and Duizer 2004).

As enteric viruses cannot replicate outside their hosts, all such virus transmission is in effect person-to-person. Environmental transit time between hosts may be brief or prolonged. Long-distance travel may take place, e.g. through water systems or even the air. Long-distance travel is accompanied by exposure to the environment and dilution; thus viruses having prolonged environmental transit times must be very stable to survive and are (usually) shed in very large numbers. Enteric viruses meet both requirements; they are acid stable and replicate to prodigious titres in the gut before being shed in concentrated doses directly into the sewage system. All potentially food-borne viruses can also be transmitted directly from person to person via faecal contamination of the environment and viewed in this way food is simply another kind of fomite in environmental transmission, it occupies a special niche simply because of its privileged position in terms of its introduction to the body and the potential it may offer for widespread distribution through trade and commerce.

The relative importance of food-borne *vs* more direct person-to-person transmission is unclear; enteric infections are ubiquitous, single occurrences are far too numerous to mention and statistics usually record only outbreaks (when several people are infected in one location or through one common vehicle). However any one outbreak may involve different types of spread; these viruses have a high secondary attack rate and person-to-person transmission will probably follow even if the virus was actually introduced to that setting by food. This can potentially mask food-borne introductions and it is likely therefore that food-borne transmission is underestimated.

3. ENTERICALLY INFECTING VIRUSES AND THEIR TARGETS

There are two types of enterically infecting virus – the first are capable of spreading elsewhere in the body. Infection by these viruses is often subclinical but they may induce signs and symptoms of disease in nonintestinal tissues. These viruses include enteroviruses (e.g. polio or Coxsackie, which may spread to the meninges, central nervous system; skeletal/heart muscle or pancreas) and hepatitis viruses A and E spreading to the liver. The second type of virus are true gut inhabitants. These replicate in the enteric tract, specific symptoms when they occur, are those of a gastro-intestinal infection; usually diarrhoea and vomiting but the extent of each component is variable.

4. THE VIRUSES

Table 1 lists the main viruses associated with enteric infection and summarizes their key properties. The most important are illustrated in Fig. 1. Enteric viruses are drawn from a variety of virus families, they range approximately 10-fold in diameter and 20-fold in terms of genome size and complexity. The major enterically transmitted and thus potentially food/waterborne agents comprise (alphabetically) the human adenoviruses (AdV), astroviruses (HAstV), caliciviruses, hepatitis E virus (HEV), parvoviruses, picornaviruses [including enteroviruses, kobuviruses and hepatitis A (HAV)], and the rotaviruses (RV). Most enteric viruses are childhood infections; spreading largely person-to-person and assisted by the lower hygiene levels in this group. Food-borne transmission may be negligible (AdV) or insignificant (RV) in the developed world. However in the undeveloped world spread of these agents by these routes is poorly characterized. Childhood infection leaves residual immunity that may prevent (or mollify) infection over the rest of an individual's lifetime. Although this is not universally true, in general viruses causing childhood illness are not significant pathogens in healthy adults previously exposed as children. The IID survey in England (FSA 2000) estimated the incidence of GP consultations for intestinal disease by patient age and causative organism. Data from this survey have been reanalysed (Fig. 2) to show the proportion of consultations made for each virus in the age groups <5 and >5 years. As expected GP consultations induced by these viruses were biased towards children <5 years; GP visits by older persons comprised only a small proportion of the total consultations. There were two exceptions to this observation; some 25–50% of GP consultations for NoV infection were made by older

Table 1 Properties of the major enterically infecting viruses

Name	Virus family (genus)	Food-borne	Size (genome)	Features	Associated illness
Polio, Coxsackie, echo, enterovirus	Picornaviridae (enterovirus)	Yes, mainly water, present in shellfish	28 nm (ssRNA)	Little surface detail Many cultivable in Vero or Hela cells	Mainly asymptomatic, can induce muscle pains (Bornholm disease), cardiomyopathy, meningitis, CNS motor paralysis
Aichivirus	Picornaviridae (kobuvirus)	Yes, shellfish	28 nm (ssRNA)	Knob-like projections Cultivable Vero cells	Gastroenteritis
Hepatitis A virus	Picornaviridae (hepatovirus)	Yes	28 nm (ss RNA)	Little surface detail Fhrk-1 cells	Hepatitis, mild in the young
Hepatitis E virus	Unclassified	Mainly water	34 nm (ssRNA)	Calicivirus-like structure Unique genetic organization Not cultivable	Hepatitis, severe in pregnancy
Rotavirus	Reoviridae	Rare often water	70 nm (dsRNA)	Multilayered Segmented genome (11 pieces) Cultivable Ma104 cells	Diarrhoea – common in the young, incidence decreasing with age, but increases in the elderly
Adenovirus group F, types 40 and 41	Adenoviridae	Not reported	100 nm (dsDNA)	Distinctive icosahedral Cultivable Graham 293 cells	Mild diarrhoea, shedding may be prolonged, mainly affects children
Saporovirus	Caliciviridae (saporovirus)	Yes (rare), mainly shellfish	34 nm (ssRNA)	Cup-like depressions on surface; none are cultivable	Gastroenteritis – common in children believed to be milder in effect
Norovirus	Calicivirus (norovirus)	Yes	34 nm (ssRNA)	Fuzzy surface structure; not cultivable	Explosive projectile vomiting in older children/young adults
Human astrovirus	Astroviridae (mamastrovirus)	Occasionally, water and shellfish	28 nm (ssRNA)	Eight serotypes 5 or 6-point star motif Appearance variable Cultivable $CaCO_2$ cells	Mostly infect children, higher serotypes seen in adults. Relatively mild gastroenteritis, but probably underestimated
Wollan, ditchling, Paramatta and cockle agents	Parvoviridae?	Yes, shellfish	25 nm (ssDNA)	Smooth featureless, poorly characterized noncultivable	Gastroenteritis – widespread shellfish-associated outbreaks, largely controlled through cooking

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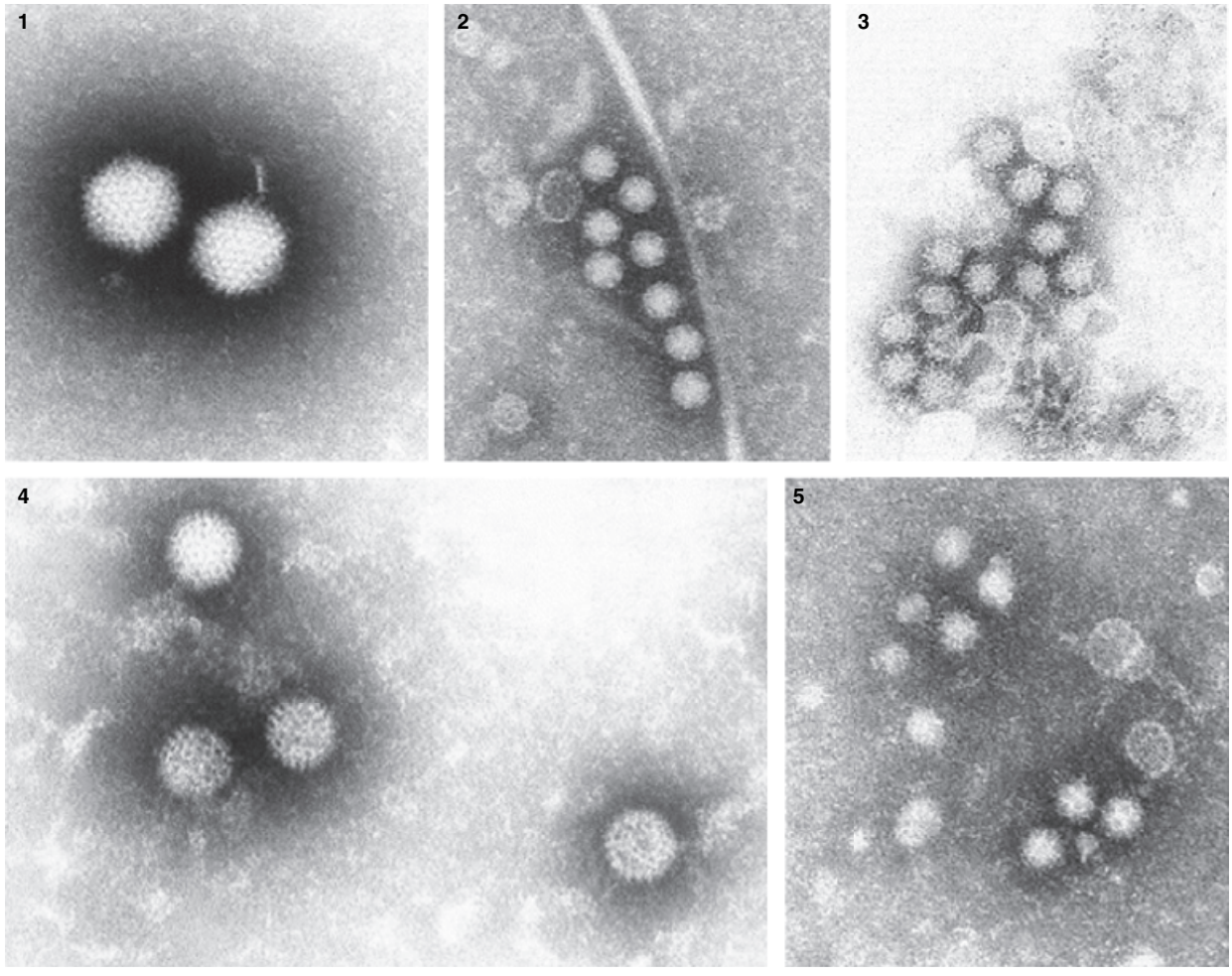

Fig. 1 Food-borne viruses. Electron micrographs of the most import enterically infecting and food-borne viruses found in clinical samples (human faeces). All panels are reproduced at the same magnification; bar represents 100 nm. Panels show: human rotavirus; 2, enteric adenovirus; 3, astrovirus; 4, norovirus; 5, sapovirus

children/adults; the highest of any virus, with astroviruses close behind.

4.1 Adenoviruses

There are 51 serotypes of AdV; all are large icosahedral DNA-containing viruses. About 30% of the serotypes are pathogenic in man; most being upper respiratory tract pathogens spreading primarily via droplets. However, even the respiratory strains grow well in the gut and are present in the faeces. Only types 40 and 41induce gastroenteritis and these are shed in larger numbers. AdV are frequently found in faecally polluted waters and have been identified in shellfish (Girones *et al.* 1995; Pina *et al.* 1998a; Vantarakis and Papapetroupoulou 1998; Chapron *et al.* 2000), but have not been appreciably associated with food-borne illness,

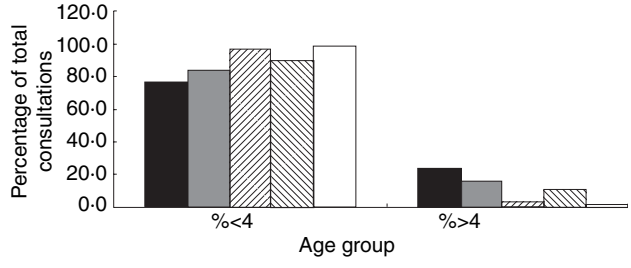

Fig. 2 Virus identifications in GP consultations for infectious intestinal disease by age of patient. Viruses identified following GP consultations have been segregated into the percentage of cases that involving children below 4 years and those involving older children and adults. Bias for infection of the young emerges clearly, even in the case of noroviruses. Data are re-analysed from FSA (2000) IID survey in England. ■, NoV; ▩, HastV; ▨, AdV; ▩, RV; □, SaV

presumably because most adults are immune and children do not commonly eat shellfish. However outbreaks of other strains associated with conjunctivitis (shipyard eye) and pharyngitis are commonly associated with exposure to polluted water, normally through recreational use (Crabtree *et al.* 1997).

Adenoviruses 40 and 41 account for 5–20% of US hospital admissions for diarrhoea, mainly in children below age 2 years (Uhnoo *et al.* 1984; Kotloff *et al.* 1989). Incubation lasts 3–10 d, and illness (usually a watery diarrhoea) may last a week. As children age, experience with AdV infection gradually increases the levels of population immunity. Only 20% of children below 6 months have antibody to these viruses, but by age 3 for this has risen to 50%. In the IID survey in England AdV infections were confined largely to children under 5 years and accounted for approx. 12% of all viruses identified (FSA 2000). Incidence was determined as 400 and 800 per 100 000 person years in the age groups 0–1 and 1–4 years. Infection is not significant in healthy adults although it may increase in significance again in the elderly (Dupuis *et al.* 1995); fewer than 4% of enteric AdV GP consultations involved persons over 5 years (Fig. 2). AdV are associated with tumours in mice but no such association has ever been made in man.

4.2 Astroviruses

Astroviruses are usually described as 28 nm rounded particles with a smooth margin. In their centres they may bear a 5 or 6-pointed 'star' motif from which they are named (Astron, a star) (see Fig. 1). However, the appearance of these agents is certainly variable, sometimes showing surface projections (Appleton and Higgins 1975) and at other times resembling caliciviruses (Willcocks *et al.* 1990). Morphology was subsequently used to present a classification scheme for many of these small viruses associated with enteric infection and including the caliciviruses (below) (Caul and Appleton 1982). Cryo electron microscopy studies have now confirmed the presence of surface projections (Matsui *et al.* 2001). Human astroviruses comprise eight serotypes (HAst1–8); types 1 and 2 are rapidly acquired in childhood; by age 7 years 50% of children are seropositive for type 1 and 75% by age 10 years (Lee and Kurtz 1982; Kurtz and Lee 1984). Exposure to the higher serotypes (4 and above) may not occur until adulthood.

Illness is generally mild, lasting 2–3 d after an incubation period of similar length. This has led many to dismiss these viruses as causative agents of significant disease in humans. However astroviruses are the second most commonly identified virus in symptomatic children (Herrmann *et al.* 1991) and account for 5% of US hospital admissions for diarrhoea – almost entirely of children (Ellis *et al.* 1984). Adults may be infected by higher serotypes and childhood antibody may not prevent clinical disease: in Japan (1995), 1500 older children and teachers were affected in a widespread food-borne outbreak of HAstV type 4 (Oishi *et al.* 1994). Finally, astrovirus identification often relies on electron microscopy but virus appearance is not always clear. Astroviruses may be frequently mistaken for small round (parvovirus)-like agents (Willcocks *et al.* 1991) and even for NoVs (Madore *et al.* 1986). In England the IID survey conducted between 1992 and 1995 (FSA 2000) found astroviruses comprised 12% of all virus identifications with incidences of 125 and 550 per 100 000 person years in the age groups 0–1 and 2–4 years respectively. However, 16% of GP consultations for astrovirus infection were made by older children and adults (Fig. 2). As this survey identified HAstV only by EM it remains possible that astrovirus infections in the adult population were underestimated. Culture conditions have been described (Willcocks *et al.* 1990) and recently an ELISA-based detection kit has been produced.

4.3 Caliciviruses

Caliciviruses appear under the electron microscope as if covered in cup-like depressions, from which the virus takes its name (*calix* = a cup). The family includes two genera that infect humans, the NoV and the sapoviruses (SaV). To date neither of these can be cultured in the laboratory. The nomenclature of these viruses has changed several times recently. Formerly they were known by names derived from their morphology (see Fig. 1): the small round structured viruses, e.g. Norwalk virus appeared fuzzy and indistinct whilst the human caliciviruses, e.g. sapporo virus, had a more obvious calicivirus-like appearance (Caul and Appleton 1982). Classification then moved to genomic organization and the groups were renamed the Norwalk-like viruses and the sapporo-like viruses. The nomenclature is now hopefully settled with the refinement of these names to the NoV and SaV respectively.

4.3.1 Noroviruses. Analysis suggests that the NoV are the single most significant cause of IID in the developed world. NoVs were first identified following an outbreak of enteric illness amongst children and adults in the town of Norwalk, OH (Alder and Zickl 1969). Although samples were first collected in 1968, viruses were not clearly identified until 1972 when antibody was used to clump the particles (Kapikian *et al.* 1972). NoVs are now routinely detected by PCR amplification of the RNA-polymerase gene and by commercial ELISA kits, electron microscopy is used as a back up. Sequence analysis of the PCR products divides the NoV into two genogroups; group 1 exemplified by Norwalk virus itself and group 2 by Hawaii virus (Lambden and Clarke 1994; reviewed in Clarke and

Lambden 2001). Recently genogroup 2 has been more common in the UK.

Infections occur around the globe and throughout the year but may be more common in winter giving rise to its former name 'winter vomiting disease'. Incubation lasts up to 48 h and is followed by a self-limiting illness lasting 24–48 h. NoV infection is not regarded as severe in otherwise healthy adults, but it is debilitating and very unpleasant. In vulnerable groups, the malnourished or elderly it can be serious and may even precipitate death. It was thought that subclinical and childhood infection was rare but recent studies have shown this does occur in very young children (Carter and Cubitt 1995). The IID study in England estimated that 1% of children < 1 years would contract NoV (FSA 2000).

Norovirus differs from other agents of gastroenteritis in three ways: first, it causes disease in adults (teenagers and above), thus NoVs are the most significant diarrhoeal virus in terms of working/education days lost. Secondly, it induces a high level of explosive projectile vomiting that may be the first obvious sign of infection. Many cases are identified at work with serious implications if a food handler should be infected. Thirdly, although there are probably multiple serotypes of NoV, immunity to all seems to be short-lived. Thus individuals may be protected for only a few months following an infection before they become infectable once more by the same virus (Parrino *et al.* 1977). Some people appear to have an inherent resistance to infection; community outbreaks that stemmed from communal exposure by swimming pool contamination showed familial clustering of symptomatic illness, and even in middle age population antibody levels are only 50%. Many of these seronegative individuals remain symptom-free and it is now thought likely that they lack the cell-surface receptor (a carbohydrate antigen) to which the virus must bind to initiate infection (Hutson *et al.* 2003). Susceptible persons require several bouts of infection by the same virus before antibody levels are boosted sufficiently to afford some protection. In the recent IID survey in England NoV accounted for 30–40% of all viruses identified, they were the most commonly identified agent in the community study, and the third most common agent that caused persons to seek consultation with their GP (FSA 2000). Several reports across the world have indicated a rise in NoV detection during 2002–03. These included shipboard outbreaks, multistate occurrences in the US, a sudden rise in outbreaks in Canada and numerous hospital outbreaks throughout the UK that forced many to close wards or cease new admissions. These have been attributed in part to the emergence of a new strain of NoV across the world characterized by mutations in the polymerase gene (Lopman *et al.* 2003, 2004). This might be more infectious than previous strains and if such an event has occurred then the mechanism underlying this process requires investigation: food-borne transmission, perhaps via international trade should be considered.

4.3.2 Sapoviruses. Sapoviruses induce symptomatic infections mainly in children. They account for some 3% of hospital admissions for diarrhoea in both the UK and US. Most children are sero-positive by age 12 and seem to become infected between 3 months and 6 years of age. SaV were found most frequently in children below age 4 in the England IID with an incidence of 460 per 100 000 person years in those aged <1 years that fell to 150 in those between 2 and 4 years (FSA 2000), <2% of GP consultations for SaV infection took place in those >5 years (Fig. 2). Infection is particularly common in institutional settings such as schools and day care centres. Incubation is between 24–48 h and illness is usually mild and short-lived with diarrhoea predominating. However in those cases when SaV have been seen to infect adults then symptoms are very similar to those of NoV (Cubitt 1989).

4.4 Hepatitis A and E viruses

Epidemic hepatitis has been recognized since ancient times, and its infectious nature was appreciated in the middle ages. However the causative agent was identified only in 1972 when the newly developed technique of immune electron microscopy permitted the particles to be identified (Kapikian *et al.* 1972). Subsequently named HAV to distinguish it from serum hepatitis (hepatitis B), this agent was found to be responsible for the bulk of infectious hepatitis. Symptoms were seen mainly in older children and adults but infection was common in younger children although in these it tended to be symptom free. HAV was found to be a member of the Picornavirus family (see below) and was initially placed in the genus *Enterovirus*. However it possessed some unique properties in relation to its genetic structure and replication procedure and it was subsequently removed to a new genus (*Hepatovirus*) of which it is the only member. HAV can be cultured in FRhK-4 cells but this is slow and difficult, especially for primary isolates.

Although responsible for most enterically transmitted hepatitis in the developed world, it was clear that, HAV alone could never account for all enterically transmitted hepatitis in the undeveloped world. Thus the concept of enterically transmitted non-A, non-B hepatitis grew up. This gap in knowledge was filled in 1990 when a new agent, HEV was identified by molecular means (Reyes *et al.* 1990; reviewed Bradley 1990, 1992). The genetic organization and particle structure of HEV resembled the Caliciviruses and HEV was initially classified in this family. However the detail of the genomic organization and the enzymic capacities encoded are such that it could not remain in this family. Consequently it

now forms the only member of a group called the 'hepatitis E-like viruses' (Green *et al.* 2000). HEV is rare in the developed world with cases generally limited to travellers.

4.5 Parvoviruses

Parvoviruses are poorly characterized as agents of enteric infection. Diagnosis is based solely on electron microscopy. Although associated clearly with gut infection in animals (e.g. canine parvovirus), the only infectious human parvovirus characterized to date is a nonenteric agent B19, causing a maculopapular rash in children. Parvoviruses have been associated with gastroenteritis in primary and secondary schools in the UK (Wollan and Ditchling agents) and Australia (Paramatta agent) (Paver *et al.* 1973; Christopher *et al.* 1978; Appleton 1987, 2001). The cockle agent was identified following a large outbreak in England (Appleton and Pereira 1977), and was associated with consumption of contaminated seafood. There is a strong argument that some/many of these viruses may actually be misidentified astroviruses, caliciviruses (Willcocks *et al.* 1991).

4.6 Picornaviruses

Picornaviruses infecting the gut were formerly all contained in the *Enterovirus* genus. Enteroviruses have a rather featureless appearance under the electron microscope and include the ECHO Coxsackie and polioviruses. Most grow well in laboratory cell cultures such as HeLa or Vero. Formerly much feared, the success of vaccination has controlled polio and allowed the WHO to target this virus for global elimination early this century. Picornaviruses were thought not to be usually associated with diarrhoeal symptoms in humans but this changed in 1993 when Aichivirus was discovered as the agent responsible for an outbreak of shellfish-associated gastroenteritis (Yamashita *et al.* 1993). Aichivirus could be grown in Vero cells and study showed that it had a genome organization typical of the picornaviruses (Yamashita *et al.* 1998). However the particle shows differences in structure from other picornaviruses and bears surface projections similar to those of astroviruses. These viruses have now been recognized as a new genus in the picornavirus family termed the kobuviruses.

4.7 Rotavirus

Rotaviruses are large RNA-containing viruses belonging to the family Reoviridae. Their particles are multilayered and complex, replicative functions may be built into the shell. Particles are readily visible and distinctive in the EM where the outer layer of the capsid can appear like the spokes of a wheel from which the virus is named (*rota*, a wheel).

Illness develops after an incubation period of 4–7 d and usually presents as diarrhoea and vomiting lasting approx. 7 d. Viruses are shed in extremely high numbers (perhaps over 10^9 per gram of stool) and diagnosis is a relatively simple matter. Virus is readily detected by direct examination by EM or PAGE (Moosai *et al.* 1984), antibody-based bead-agglutination or ELISA systems. RV account for some 3·5 million cases of diarrhoea p.a. in the US equating to 35% of hospital admissions for diarrhoea (Ho *et al.* 1988). Approximately 120 children die each year in the US from this virus and fatalities in the undeveloped world may amount to millions (Parashar *et al.* 2003).

Rotaviruses occur in five groups (A–E) but only groups A–C infect humans. Group A is by far the most common with sporadic episodes due to group C, group B is limited largely to China. Only group A viruses can be cultured, these are grown in Ma104 cells. Within each group RV are divided into serotypes based on their surface-exposed proteins. Within group A there are 14 types of VP7 (termed G types) and approx. 20 of VP4 (termed P types). This generates great antigenic diversity permitting serial infections which may be symptom free. The peak age for illness is between 6 months and 2 years, by 4 years most persons have been infected. Immunity to rotavirus is long-lasting, thus sequential exposure leads to accumulated immunity and frequency of illness decreases with age. Silent secondary re-infections can occur (as in parents caring for infants) and this provides another means for the virus to spread in the community. RV were the most commonly identified enteric pathogen in children <4 years in England and Wales and comprised 25–35% of the virus identification made in this study (FSA 2000). Only 11% of GP consultations for rotavirus were made by persons >5 years. However this raises two points, first not all adult infections are mild and secondly this small percentage may actually reflect a greater number of adult GP consultations for rotavirus infection than for NoV (FSA 2000).

5. PATHOGENICITY OF FOOD-BORNE VIRUSES

5.1 Gastroenteritis

Gastroenteritic viruses replicate and destroy the mature enterocyte covering the upper third of the intestinal villi. Undifferentiated, immature cells do not support virus replication. Destruction of functional mature cells disrupts the reabsorption of water from the gut and diarrhoea ensues. The villi retract in response to damage and decrease the surface area available for absorption. At the same time the crypt cells undergo rapid division and soon repopulate the villi with young, as yet undifferentiated cells. These immature cells are resistant to virus infection but cannot

replace the function of those that have been lost; they require time to mature. Thus malabsorption continues until the cells can develop the necessary ion uptake capabilities. This exuberant cell division in the crypt is central to recovery. Fortunately in humans viruses do not attack the crypt cells themselves. When such infections do occur (e.g. canine parvovirus) they result in bloody diarrhoea from which recovery may not be possible (especially in a young animal).

5.2 A novel virus toxin

Although most viruses do not manufacture toxins some RV are exceptional and induce the synthesis of a toxin-protein termed NSp4 that can induce diarrhoea if administered alone (Ball *et al.* 1996). NSP4 has no similarity to bacterial toxins (Tian *et al.* 1995). It stimulates transepithelial chloride secretion via a calcium ion-dependent path; it has no effect on cAMP formation and is independent of the cystic fibrosis channel. The mechanism involves stimulation of inositol production (Dong *et al.* 1997). The protein also has direct effects on brush border transport mechanisms (Halaihel *et al.* 2000). It is proposed that at least one form of soluble NSp4 is released from infected cells and binds to neighbouring cells (Zhang *et al.* 2000).

5.3 Hepatitis

Hepatitis A and E viruses enter via the gut and may replicate there, however both move rapidly to the liver and invade the hepatocytes. Clinical features of both viruses are similar although HEV is more severe and may have a fatality rate of 20–40% in late pregnancy. HEV has a longer incubation period than HAV (60 d *vs* 48 d) and a more prolonged viraemia (Clayson *et al.* 1995b; Scharschmidt 1995; Reid and Dienstag 1997). The long incubations make identification of the source of infection problematic as contaminated food will usually have been eaten or disposed of before illness arises. Haemoglobin breakdown by the liver is impaired and a bilirubin (normally shed in the bile and thence in the faeces) overflows into the blood. The skin and whites of the eye turn yellow (jaundice) and faeces become pale. Bilirubin is filtered from the blood by the kidneys, and urine becomes dark. Virus particles are shed into the bile and thence in the faeces, but in contrast to the cell destruction caused by gastroenteritis viruses there is little virus-induced liver cell damage. HAV interferes only weakly with host cell activities and new viruses are released inside membrane-bound packets without necessity to lyse the cell. An immune response develops 2–3 weeks after infection and leads to immune attack on infected liver cells. It is this host response rather than the virus itself that causes the signs of liver damage. Eventually the immune response eliminates all infected cells (and thus the virus) from the body. Convalescence may be prolonged (8–10 weeks) and some 15% of HAV cases may follow a relapsing course over 12 months or more.

Hepatitis viruses A and E have been affected by human activities. In former times, infection occurred early in life, often whilst still protected by maternal antibody. Such endemic infections tended to be mild or subclinical. Both viruses are rare where sanitation is improved. This has reduced exposure and so increased the age at which first infection occurs; in Hong Kong, 30% of those under 30 were seropositive in 1979; by 1989 this had fallen to only 9%, although seropositivity in the elderly remained high. In contrast, in France where sanitation has been good for many years, 80% of persons over 30 have no antibody to HAV (i.e. have never been infected).

This shift in age at infection increases the severity of infection: below 3 years, HAV infection is virtually always subclinical; but symptomatic infections predominate by 5 years and severity worsens with age (Hadler *et al.* 1980). Persons over 50 years of age account for only 12% of the cases of HAV but have a case-fatality rate sixfold higher than average (CDC 1994; Fiore 2004). This delay in infection allows a pool of susceptible individuals to accumulate in the community and establishing conditions for epidemic spread. Analysis of annual incidence figures in the US reveals evidence of epidemic behaviour (Fiore 2004). The Centers for Disease Control, USA estimates 267 000 cases occurred on average per year between 1987 and 2001, most were mild or symptom free but 10–30 000 acute cases were registered annually. Mead *et al.* (1999) estimate that 5% of cases are food-borne. The situation in the UK has been summarized (Crowcroft *et al.* 2001). The existence of susceptible adults in some parts of the world is significant in the context of food-borne infection since trade that could bring virus-contaminated food grown/produced in areas of high endemicity to areas of low prevalence could pose a threat for adults in those areas (see below).

Hepatitis E virus is not significant in the UK or US; most infections are limited to returning travellers. Epidemics of HEV are known, often spread by contaminated water; the worst cases involved 30 000 people in New Delhi (1955); 100 000 in Xinjiang Uighar, China (1986), and 79 000 in Kanpur India (1991) (Grabow *et al.* 1994; Scharschmidt 1995). More limited shellfish-associated outbreaks occur sporadically around the Mediterranean. HEV replicates in pigs (Balayan *et al.* 1990) and has been found in both wild and domestic cows, goats and pigs (Clayson *et al.* 1995a). Replication also occurs in laboratory rats (Maneerat *et al.* 1996; Meng *et al.* 1996). These findings mean that animals might act as reservoirs for infection (Kabrane-Lazizi *et al.* 1999; Wu *et al.* 2000). Seropositivity has been estimated at 2–10% even in areas free from human disease; 15% of homeless persons in Los Angeles revealed antibody to HEV, possibly through contact with infected urban rats (Smith *et al.* 2002). Similarly

HEV has been detected in sewage from areas in which clinical disease is absent (Pina *et al.* 1998b).

6. VIRUS STABILITY

The stability of an enteric virus is of fundamental significance to its transmission. First, it may limit the period for which virus contamination remains a threat in the environment whether in sea or fresh water or dried onto a surface, and secondly, it governs the efficiency of attempts to deliberately destroy a virus, e.g. in food or water processing (mainly sensitivity to temperature and chlorination). Many enteric viruses are nonenveloped and contain RNA as their genetic material (exceptions AdV and parvovirus that contain DNA). RNA is labile, hydrolysed at both acid and alkaline pH and destroyed by radiation or enzymic processes. DNA is more stable but is still sensitive to UV irradiation. This emphasizes the crucial role of the virus coat proteins that must protect the nucleic acid. The relative stabilities of some of the enteric viruses are assembled from a variety of sources and presented in Table 2. There is no central data set for all viruses and their frequently used surrogates; most studies use only a few viruses under restricted conditions and have not considered the effects of combined treatments. The environment in which inactivation proceeds will certainly affect the values determined and this has central significance when considering survival in different foodstuffs (e.g. inside shellfish or in milk) or in the environment (marine *vs* fresh water etc.). As each study has used slightly different conditions, this table should be regarded as only indicative.

Although all these viruses are destroyed by boiling, the thermal stability of some is remarkable. Temperatures above 90°C are required to inactivate HAV (Millard *et al.* 1987) and others suggest 100°C may be necessary (Croci *et al.* 1999). Inactivation is often biphasic and a residue of resistant virus may survive. This probably represents virus in a protected microenvironment, e.g. aggregated or combined with materials exerting a protective/stabilizing effect (Tierney and Larkin 1978; Larkin and Fassolitis 1979; Bidawid *et al.* 2000a).

Virus persistence in dried matter depends on the surface onto which it is dried, the presence of extra (faecal) material and the temperature/humidity of storage. Many enteric viruses survive for long periods on common surface types requiring up to 60 d for a 2 log reduction in titre (Abad *et al.* 1994a). In general reducing the temperature and adding faecal contamination promotes virus survival but viruses may respond differently to relative humidity; HAV and rotavirus are stabilized at low relative humidity whilst enteroviruses are stabilized at higher values (Mbithi *et al.* 1991). This probably depends on the type of surface onto which the viruses are dried (Abad *et al.* 1994a).

Values for inactivation times/persistence in water vary widely with reported T90 values (i.e. time for 1 log titre reduction or 1 TLR) for enteroviruses of 14–288 h. A 4 log reduction times in sea water are likewise variable but are probably measured in weeks (Chung and Sobsey 1993; Callahan *et al.* 1995). Persistence in artificially contaminated water has been demonstrated for >1 year (rotavirus and poliovirus) (Biziagos *et al.* 1988) and 300 d for AdV 41 (Enriquez *et al.* 1995). These variations probably reflect differences in the conditions used particularly, type of water, illumination, turbidity and pH, and emphasize the need for standardized studies of all relevant viruses (and common surrogates) under directly comparable conditions. Despite this variation all these viruses are probably capable of survival for weeks/months at environmental temperatures and at low temperatures, sheltered from UV irradiation some may persist for years.

7. WATERBORNE VIRUS INFECTION

All these viruses enter the sewerage system and may survive wastewater treatment to contaminate receiving waters. Here they could pose a threat to recreational users or to consumers of shellfish or other produce eventually destined for the food chain. In addition, where such waters are later abstracted and treated for use as drinking water, contaminating viruses might survive this treatment too. Drinking water can also be contaminated after treatment if supplies are not adequately separated from untreated sources.

7.1 Wastewater treatment and virus survival

Enteric viruses are usually shed in large numbers, rotavirus, titres can exceed 10^9 particles per ml and could comprise up to 2 mg in every gram of stool. A value between 10^6–10^8 per ml is common for astrovirus and AdV. Caliciviruses, entero- and hepatitis viruses are often shed in lower (but still appreciable) numbers; cultivable enteric viruses are ubiquitous in human populations and levels in sewage can exceed 10^4 PFU per litre. Water treatments lacking a tertiary step (e.g. UV treatment) reduce this load only poorly. Sewage sludge production reduces numbers by 95% but many viruses survive giving levels in receiving waters of up to 100 PFU per litre if contamination is serious and 1–10 PFU per 100 l where it is less so (Gerba *et al.* 1985; Bloch *et al.* 1990; Jothikumar *et al.* 2000; Scipioni *et al.* 2000; Pina *et al.* 2001). Most enteric viruses have been detected in wastewater, treated water and receiving waters over time, usually by PCR techniques even if viruses are cultivable, e.g. HAV (Dubrou *et al.* 1991; Goswami *et al.* 1993; Graff *et al.* 1993; Tsai *et al.* 1993; Jaykus *et al.* 1996; Schwab *et al.* 1996) and astrovirus (Le Cann *et al.* 2004). AdV have consistently been detected in raw sewage and approx. 80% may be enteric

Table 2 Virus stability values; assembled from references below. No study has included all these viruses under identical conditions and thus data are not always comparable; values presented here should be taken as indicative of stability only. Log titre reduction (LTR), measured by infectivity unless otherwise stated. Bacteriophage MS2, a common surrogate for enteric viruses is included for comparison

	Persistence/inactivation conditions							
Virus	pH stability	Thermal inactivation	Free chlorine (unless stated)	UV irradiation (mJ cm^{-2})	Persistence (dry)	Persistence food matrices	Persistence sea water	Persistence fresh water
Adenovirus	Stable pH 6–9·5*	Inactivated 56 deg, 10 min* (6)	2 LTR at 0·5 mg ml^{-1} for 10 min (20) 3 LTR at 1·0 mg ml^{-1} for 10 min (20)	4 LTR at dose 216 (23)	2·5 LTR on drying, further 3 LTR 10 d (19)	Not significantly food-borne	1 LTR in 40 d 15 deg (27)	3·2 LTR in 60 d at 20 deg 1 LTR in 40 d at 15 deg (27)
Astrovirus	Resists pH 3 (4)	Resists 50 deg 1 h, 60 deg 5 min (4)	2 LTR at 1 mg l^{-1} for 10 min, 2·0 LTR at 0·5 mg l^{-1} for 20 min (18)	No data	1 LTR on drying then stable for 60 d at 4 deg (19)	No data	Reportedly lower stability than fresh water	Drinking water: 2 LTR at 4 deg, 3·2 LTR at 20 deg both 60 d
Hepatitis A virus	Resists pH 1·0 for 2 h (9)	Resists 60 deg for 10 min; cations stabilize to 81 deg Inactivated 98–100 deg (7)	Inactivated by 2–2·5 mg l^{-1} for 15 min (13) CT for 99% reduction = 8 (17) 1 LTR at 0·5 mg l^{-1} for 10 min; 2 LTR at 1 mg l^{-1} for 10 min (20); 3 LTR at 4 ppm ClO_2 for 10 min (16)	4 LTR at dose 16–39 (23); 1 LTR at dose 36·5 (30); 1 LTR in 1·3 min at 42 mW cm^{-2} in sea water (25)	Survives >1 month at 25 deg in 42% humidity (10, 11); 3 LTR in 8 d and then stable to 32 d (16)	Resists 60 deg for 19 min in oysters (12) 2 LTR at 63 deg in water or milk for 60 min (16) 2 LTR in 7 d in creamy wafer cookies 21 deg	2 LTR in 28 d at 25 deg (11)	Estuarine water: 2 LTR in 28 d at 25 deg (11) PBS: 1 LTR in 56 d at 25 deg
Norovirus	Resists pH 2·7 3 h (14)	Resist 60 deg for 30 min (14)	Resists exposure to 6·25 mg l^{-1}; 30 min (CT 187·5) inactivated at 10 mg l^{-1} (CT 300) (15); others suggest CTs 30–60 required (see text) 1 LTR (PCR) at 2 mg l^{-1} chloramine for 3 h (25)	No data – FCV surrogate T90 value 47·85 (30)	NoV Recoverable from disinfected hospital surfaces; FCV 2 LTR in 15 d at 4 deg; or in 30 d at 20 deg, inactivated in 1 d at 37 deg (31)	Infectivity in shellfish not reduced after 1 month storage at 4 deg or over 4 months frozen. Stable in ice	1 LTR in 24 h at 10 deg (illuminated)	FCV suspension – 1 LTR per day at 37 deg, 4 LTR in 60 d at 4 deg (31)

Table 2 (*Continued*)

Virus	Persistence/inactivation conditions							
	pH stability	Thermal inactivation	Free chlorine (unless stated)	UV irradiation (mJ cm^{-2})	Persistence (dry)	Persistence food matrices	Persistence sea water	Persistence fresh water
Picornavirus/ enterovirus	Resists pH 3·0 (7)	Destroyed 42 deg, cations stabilize to 50 deg(8)	2 LTR at free chlorine 1·1–2·5 mg l^{-1} (28) 1 LTR by chloramine, 2 mg l^{-1} for 3 h (25)	1 LTR in 1·3 min at intensity 42 mWs cm^{-2} in sea water (25) 1 LTR at dose 24·1 (30)	2 LTR on drying, further 3 LTR in 60 d at 4 deg	>3 LTR in water or milk at 63 deg, 30 min	1 LTR in 96 h at 12 deg; 1 LTR in 22 h at 22 deg (22); 1 LTR >670 d at 4 deg (26)	1 LTR 24–31 h in river water *in situ* 12–20 deg (21)
Picornavirus/ kobuvirus	Resists pH 3·5 (7)							
Rotavirus group A	Stable pH 3–9 (5)	Resists 50 deg (3)	CT values for 2 log inactivation 0·01–0·05 (17) Group B virus: 1·8 LTR 10 min 1 mg ml^{-1}; 1·5 LTR 10 min 0·5 mg l^{-1} (20)	4 LTR at 56 (23)	1 LTR on drying; 1 further LTR in 30 d 4 deg (19)		Bovine Rotavirus – decay 0·5 LTR per day natural sea water (29)	Group A virus: 2 LTR >64 d tapwater or 10 d river water 20 deg (27); Group B virus: 3·2 LTR in 60 d 20 deg (1, 2)
MS2	Tolerates low pH	>1 LTR in 15 s at 72 deg in water or milk (16)	1 LTR by monochloramine 2 mg l^{-1}, 3 h (25) >3 LTR by 10 min in 2 ppm CLO2 (16)	4 LTR at dose 750 253 nm (24) 1 LTR dose 23·04 (30)	App 3 LTR in 4 d (16)			

Data sources: 1. Vondefecht *et al.* (1986); 2. Terrett *et al.* (1987); 3. Estes (1991); 4. Monroe *et al.* (2000); 5. Mertens *et al.* (2000); 6. Russel *et al.* (1967); 7. King *et al.* (2000); 8. Dorval *et al.* (1989); 9. Scholz *et al.* (1989); 10. McCaustland *et al.* (1982); 11. Sobsey *et al.* (1988); 12. Peterson *et al.* (1978); 13. Coulepsis (1987); 14. Dolin *et al.* (1972); 15. Keswick *et al.* (1985); 16. Miriam and Cliver (2000); 17. Hoff (1986); 18. Abad *et al.* (1997); 19. Abad *et al.* (1994a); 20. Abad *et al.* (1994b); 21. O'Brien and Newman (1977); 22. Bitton (1978); 23. Cotton *et al.* (2001); 24. Someer *et al.* (2003); 25. Shin and Sobsey (1998); 26. Gantzer *et al.* (1998); 27. Enriquez *et al.* (1995); 28. Clark *et al.* (1993); 29. Loisy *et al.* (2004); 30. Nuanualsuwan *et al.* (2002); 31. Doultree *et al.* (1999).

*Nonenteric virus strains.

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forms. These appear to survive sewage treatment well and are frequently detected even in waters that lack enterovirus contamination. This has led many to suggest that AdV may be appropriate sentinels for the indication of faecal pollution in water (Enriquez *et al.* 1995; Pina *et al.* 1998a; Wyn-Jones and Sellwood 2001).

Noncultivable viruses have also been detected. NoV have been found in sewage influent and effluent waters using PCR techniques (Lodder *et al.* 1999), serial dilution of influent waters suggested that levels may exceed 10^7 detectable virus units although this cannot be readily correlated with particle numbers or infectivity (Wyn-Jones *et al.* 2000). Estimates using quantitative PCR obtained similar values (Laverick *et al.* 2004). A study of treated water suggested that NoV levels were virtually undiminished in primary treatment and were reduced by only a little over 1 log in the final (secondary) effluent (Cross 2004). Similarly astroviruses have been detected in sewage treatment plant inlet and effluent waters, indicating a reduction of approx. 2 log during processing; effluent waters still contained 10^5 detectable astrovirus genomes per litre (Le Cann *et al.* 2004). HEV was found in sewage by molecular means (Pina *et al.* 1998b), and survives at least some wastewater treatments although its removal has not been quantitated (Jothikumar *et al.* 1993).

Viruses surviving water treatment and entering receiving waters could persist and pose a risk for recreational users of this water and studies have inferred potential exposure to both NoV (Gray *et al.* 1997) and HAstV (Myint *et al.* 1994) from this source. Studies have indicated that the risks of disease resulting from exposure to recreational water may be as high as 1/1000 for AdV (Crabtree *et al.* 1997) and perhaps higher for rotavirus (Gerba *et al.* 1996).

The mechanisms of virus inactivation/removal during wastewater treatment are not clearly understood and the environment itself probably alters the effectiveness of each process. Physical removal seems to be significant and much virus (perhaps 95%) appears to be removed via activated sludge or complexing with mineral particles. Active enterovirus has been recovered from sludge (Albert and Schwarzbrod 1991) and HAV was detected by both antigen capture and PCR suggesting that particles were intact (Graff *et al.* 1993). EU requirements for heat-treatment before sludge is spread onto land should control virus contamination from this source if followed [EU directive on sewage sludge in agriculture (86/278/EEC) implemented under the sludge (use in agriculture) regulations 1989] but there may be fewer controls in the less developed world and these might contaminate crops destined for export.

7.2 Potable water treatment and virus survival

Tap water may account for some 14–40% of gastrointestinal illness and thus efforts to ensure its safety are vital (Payment 1988; Payment *et al.* 1997). The assurance of drinking water quality begins with the source water, which should be obtained from sources as far removed from potential contamination as possible. In the US subsequent treatment aims to reduce levels of contaminating virus by 99·99%. Filtration can achieve an initial 10-fold reduction with a further 1000-fold achieved by active disinfection (e.g. chlorine, chlorine dioxide, ozone or UV irradiation). Of these, chlorination is the most widespread; peak levels are usually around 1 mg l^{-1} for 60–240 min (Grabow 1990; Thurston-Enriquez *et al.* 2003) and water entering the US distribution system should have a residual level of 0·2 mg l^{-1} to comply with requirements to control coliform bacteria. Exposure to disinfectant is expressed by the contact time (CT) parameter, concentration of agent (mg l^{-1}) multiplied by time (min). CT values for a 4 log inactivation of enteric viruses fall in the range of 4–400 mg·min l^{-1} at a contact concentration of 0·4 mg l^{-1} at 5°C. However the rates of virus destruction and thus CT values required are temperature dependent, doubling for every 10°C rise and potentially defective around 0°C. Increasing the pH from 6 to 9 reduces the efficiency of free chlorine threefold, ozone or chlorine dioxide are unaffected. Turbidity has the greatest effect, shielding viruses from UV radiation, promoting aggregation and also 'mopping up' virucide. Increasing the turbidity from 1 to 10 Nephelometric Turbidity Units decreases free chlorine effectiveness eight times (LeChavallier *et al.* 1981; Hoff 1986; HECS 2003). Thurston-Enriquez *et al.* (2003) re-evaluated the efficiency of chlorine and found viruses to be 30 times more resistant when aggregated. If this is so then procedures commonly in use in the US should destroy most but possibly not all aggregated viruses (Thurston-Enriquez *et al.* 2003).

Norovirus was found relatively insensitive to chlorine; in volunteer studies the virus survived exposure to 6·25 mg l^{-1} for 30 min (CT 187·5) and required 10 mg l^{-1} for destruction (CT 300 mg·min l^{-1}) (Keswick *et al.* 1985). These values imply that NoV could survive some water chlorination procedures. However, these experiments are difficult to do and rely on clinical samples as NoV cannot be cultivated. Keswick *et al.* (1985) used a crude sample of very high titre that may well have protected the virus. A protective effect due to such factors was later noted (Meschke and Sobsey 2002). When purified, NoV has the same sensitivity to chlorine as poliovirus (Shin and Sobsey 1998; Meschke and Sobsey 2002). A PCR-based study found a CT value of 30–60 to be sufficient to inactivate the virus (Shin and Sobsey 1998). However it is difficult to determine which of these findings is the more relevant as viruses are not found 'pure' in the environment, or even in the same form in all locations; exogenous material and aggregates are always likely to be present. Rotavirus is inactivated efficiently by chlorine (CT 112·5 at 3·75 mg l^{-1}) and for

this virus waterborne infections in the developed world are usually linked to contamination of 'clean' water post-treatment (e.g. Hopkins *et al.* 1984).

Enteric viruses have been found in drinking water (1–20 PFU per 1000 l) (Payment 1988; Gerba and Rose 1990; Payment *et al.* 1997). Studies in France showed that the presence of astrovirus RNA in tap water was correlated with an increased risk of intestinal disease (Gofti-Laroche *et al.* 2003). However it is not at all clear what an acceptable level of virus contamination might be; a risk of infection of 1×10^{-4} per person per year has been accepted in both the US and the Netherlands (USA EPA 1991; Staatscourant 2001). Using a model based on rotavirus dose–response data, Regli *et al.* (1991) calculated that $2{\cdot}22 \times 10^{-7}$ viruses l^{-1} would be consistent with this risk factor. A 4 log reduction achieved from even highly rotavirus-contaminated source water (Raphael *et al.* 1985) would meet this level (Gerba *et al.* 1996). Estimates based on enterovirus levels suggest that higher reductions might be required (Regli *et al.* 1991). Such assessments are variable, poor culture efficiency would underestimate the levels present and increase risk to the consumer; uneven distribution of contamination would cause an uneven risk across the country and differences in levels of consumption (and the proportion that is boiled before drinking) also affect risk. Finally models based on infection may be less relevant than models based on symptomatic illness and could thus exaggerate the risk (Gerba *et al.* 1985; Gale 1996; Payment *et al.* 1997; Haas *et al.* 1999; Hurst *et al.* 2001).

Most documented instances of drinking water contamination by viruses have been attributed to contamination of previously 'clean' water. This can result simply from contamination of drinking water supplies, e.g. through heavy rainfall or flood overwhelming treatment works and permitting untreated or partially treated sewage to contaminate wells (Cannon *et al.* 1991; Kukkula *et al.* 1997, 1999). Failures in treatment processes themselves can also allow contamination, e.g. pressure failure (Kaplan *et al.* 1982), insufficient disinfection, or exceptionally high levels of virus overwhelming a correctly applied procedure (Payment 1988; Gerba and Rose 1990; Payment *et al.* 1997; Bitton 1999; Gofti-Laroche *et al.* 2003). Virus leakage from sewers or septic tanks can contaminate clean water (Hedberg and Osterholm 1993). Waterborne outbreaks of enteric virus infection are common; between 1980 and 1994 the US recognized 28 outbreaks and 11 195 cases of waterborne illness, of these 9000 cases were NoV and nearly 400 HAV (HECS 2003). None of these viruses are appreciably affected by freezing (which may actually preserve infectivity for long periods) and thus infection is also spread when ice is manufactured and distributed using contaminated water (Cannon *et al.* 1991).

8. FOOD-BORNE VIRUS TRANSMISSION

Food-borne transmission may be divided into two areas: in primary contamination food materials are already contaminated before they are harvested, e.g. shellfish grown in contaminated waters, or soft fruits irrigated/sprayed with contaminated water. Secondary contamination occurs at harvest or during processing and emphasizes the role of the food handler preparing foods for others with whom he/she does not come into direct contact. Food handlers in this context include field harvesters, production plant workers right through to professional chefs and caterers. Contamination from these persons involves not only transfer of virus from infected persons but also their use of polluted water or materials in processing. It is worth noting that transfer of virus from an infected individual to food would be counted as a food-borne infection if it takes place in the commercial/industrial setting, but as person-to-person infection if it occurs in the home.

It is difficult to assess the extent of food-borne transmission in the community, first because person-to-person and food-borne routes may overlap (above), but secondly because it is technically difficult to detect viruses in foods, apart from shellfish most are not routinely tested. This has led to two assumptions, first most food-borne outbreaks in which a vehicle is not demonstrated are likely to have arisen by contamination from food-handlers – at or close to the point of serve – and the second is that most (if not all) cases occurring in the community (i.e. outside outbreak situations) probably arise from person-to-person transmission. These assumptions should be reconsidered because both disguise or negate potential instances of primary or distant secondary contamination.

Estimates of the incidence of food-borne disease have relied on statistics obtained from outbreaks. In recent years, reports of viral gastroenteritis, particularly NoV outbreaks have shown a dramatic increase in England and Wales; 418 cases were identified in 1992 and 2387 in 1996. In 1994 UK reports of NoV outbreaks outnumbered those of *Salmonella* for the first time. This increase may be partially explained by increased awareness and improvements in virus identification techniques, thus more recently attempts have been made to estimate the extent of community illness. Mead *et al.* (1999) estimated that 67% of food-borne illness in the US was caused by viruses and that 40% of NoV infections; 1% astrovirus, 1% rotavirus and 5% of HAV infections, respectively, were food-borne. NoVs were responsible for 23 million cases per year with over 9 million food-borne events. NoV then emerged as the most significant food-borne infection in the US. Annually NoV was estimated to be responsible for 20 000 hospitalizations and 120 deaths.

8.1 Shellfish

Enterically transmitted viruses are shed into sewage and become rapidly diluted as they travel through the water system. Filter-feeding molluscan shellfish (particularly cockles, mussels and oysters) partially reverse this dilution because they are filter-feeders. They are harvested from close-to-shore locations where water will contain virus inputs from sewage effluents; viruses become concentrated within the shellfish and may be retained for some time. Levels of virus in shellfish may be 100–1000-fold higher than concentrations of viruses in their surrounding water.

European regulation divides shellfish harvesting waters into different categories depending on the levels of faecal pollution (Table 3). Very badly contaminated waters may not be used to harvest shellfish for human consumption at all, whilst intermediate levels may be used provided that the shellfish are relayed into cleaner waters, heat-treated or depurated before harvest. Depuration is a process in which the animals are kept in a recirculation tank; water is passed over the shellfish, disinfected by UV treatment and recycled. The shellfish gradually purge their bodies of faecal indicator bacteria and may be sold when these reach a target level. It is now clear that bacteria are not an adequate indicator of the presence of enteric viruses, and numerous virus infections have been documented from shellfish compliant with these regulations (Chung *et al.* 1996; Griffin *et al.* 1999; reviewed by Lees 2000). Bacteria are purged more rapidly than viruses. Schwab *et al.* (1998) found that a 95% reduction in *Escherichia coli* was achieved by 48 h depuration but NoV was reduced by only 7% in the same period. The efficiency of virus removal is influenced by a number of factors chiefly temperature (Dore *et al.* 2000). Most gastroenteritis viruses are more common in winter (even if not shellfish transmitted) and increased community illness coupled with less efficient purging by the shellfish could increase the risks associated with shellfish consumption at these times. Viruses retain infectivity well in shellfish and no loss of infectivity was observed over 1 month in refrigerated storage (Tierney *et al.* 1982) or 4 months when frozen (Di Girolamo *et al.* 1970).

Trade in shellfish permits long-distance transmission: in 2002 oysters from Cork Bay spread NoV infection around the world to Hong Kong (ProMED-mail 2002a; 20020331.3850), and in 2004 Chinese frozen oysters were implicated in a (presumed NoV) outbreak in Singapore (ProMED-mail 2004; 20040107.0075). In 1993 a multistate outbreak of NoV illness occurred in the US affecting up to 186 000 people (Berg *et al.* 2000). This was traced to contaminated oysters harvested in the Gulf of Mexico. Oysters in Europe accumulate a mixture of all viruses present in their environment, but in these Gulf oysters only one strain was present. This indicated that input virus must have originated from a restricted number of infected persons and was possibly attributable to incorrect disposal of faeces or vomitus at sea. This remarkable occurrence indicates firstly just how efficiently the shellfish can accumulate virus from their environment and secondly just how significant commercial trade and distribution can be in the dissemination of infection. It further demonstrates the power of molecular epidemiology in linking occurrences that would otherwise not necessarily have been connected. Sporadic outbreaks occur throughout the developed world via consumption of virus-contaminated shellfish (reviewed by Lees 2000).

Many shellfish are subject to minimal cooking (if any), linking this to their known ability to concentrate environmental viruses the fact that they are the most commonly identified source of food-borne virus infection should not be surprising. The most significant viruses in this context are NoV first reported as an Australia-wide outbreak (Murphy *et al.* 1979), and hepatitis viruses. Recently Aichivirus has been associated with infection from this source in Japan but reported cases are few. However the viruses most commonly detected in shellfish are enteroviruses (reviewed by Gerba and Goyal 1978). These seldom give rise to symptomatic disease although some cases have been reported (Cliver 1997).

Virus concentration by shellfish is nonspecific and thus their consumption can expose the consumer to a cocktail of viruses that might have different effects when present together. Limitations on current technology mean that during investigations is it usual to seek only a few defined virus types (those that succeed in inducing clinical disease). However the possibility of multiple simultaneous infection brought about by the consumption of shellfish should not be overlooked, viruses might act synergistically to increase the

Table 3 Microbiological classification of shellfish harvesting waters and requirements for marketing for human consumption (taken from EC directive 91/492). Virus contamination is not assessed

Class	Standard	Requirements for marketing for consumption
Class A	<230 *E. coli* or 300 faecal coliforms	May be consumed directly
Class B	90% compliance <4600 *E. coli* and 600 faecal coliforms	Depuration or relaying until class A standard is met, or apply heat-treatment
Class C	<60 000 faecal coliforms	Relay for 2 months to meet class A or heat-treat when class B standards met. Or heat-treat
Prohibited	>60 000 faecal coliforms	Prohibited

severity of symptoms or to present confusing clinical pictures.

Shellfish transmission of HAV is well documented and includes the largest ever outbreak that of Shanghai (1988); 300 000 persons were believed to have been infected through consumption of contaminated clams (Halliday *et al.* 1991). Studies around the world have consistently identified shellfish consumption as a major risk factor for the contraction of hepatitis. In some studies the risk was equivalent to that of contact with an infected person (Koff *et al.* 1967; Kiyosawa *et al.* 1987). HAV is rarely detected in food itself, largely because of the long incubation period for this illness which means that no samples of suspect food are likely to be available for testing. Even so shellfish are believed to be the major vehicle for HAV infection and Salamina and D'Argenio (1998) estimated that up to 70% of all hepatitis A in Italy was contracted from shellfish. In the UK it is recommended that shellfish flesh be raised to 90°C for 1·5 min (Millard *et al.* 1987) and continuous flow methods now ensure that all shellfish are subjected equally to this treatment. Since the implementation of these recommendations, there have been no reports of outbreaks in the UK of either viral gastroenteritis or hepatitis A associated with shellfish heat-treated in this way (Appleton 2000). Work by Croci *et al.* (1999) suggested that even these standards may not be sufficient to completely eliminate the virus and they recommended treatment at 100°C for 2 min.

Hepatitis E is not commonly transmitted by shellfish (Chan 1995; Stolle and Sperner 1997) but has been identified in sewage even originating from nonendemic areas (Pina *et al.* 1998b). This finding may be related to the seroconversions also identified in such nonendemic regions (see above). Thus the possibility of shellfish transmission must be considered.

There are few reports of astrovirus transmission via shellfish consumption (Caul 1987) although recently shellfish were implicated in a large outbreak involving teachers and young adults at a school in Japan (Oishi *et al.* 1994). This was unusual in that it affected older persons and was attributed to a higher serotype virus. Astroviruses are frequently detected when sought in oysters and the apparent lack of resultant illness is presumably attributable to prior immunity remaining from childhood infection. Similar considerations apply to rotavirus contamination.

8.2 Soft fruit and salad vegetables

After shellfish, food-borne virus illness is most commonly linked to salads and soft fruits. These items are almost always subject to handling immediately before serving and this presents an opportunity for contamination. In virtually all cases where this type of food is implicated an infected food handler is suggested as the origin but in only a small proportion of such cases is this link actually proved. Soft fruits and salad vegetables, like shellfish are eaten raw but also share other features; first, they all have a high water content – absorbed from groundwater during growth; secondly, many are eaten without peeling which would remove external contamination. Consequently, primary contamination is possible externally by spraying or internally by uptake of viruses from contaminated irrigation water or fertilizer. Surface splash may be significant for fruits close to the ground (e.g. strawberries) and multi-state outbreaks of HAV may have arisen in this way (Niu *et al.* 1992; Hutin *et al.* 1999). Wastewater may be used for irrigation, especially in dry areas where water is more precious, and these communities show elevated incidence of hepatitis virus infection although reasons have not been identified (Katzenelson *et al.* 1976).

Viruses can enter plants through root damage (Katzenelson and Mills 1984), and this occurs universally through root abrasion with soil particles. Other workers found virus uptake from roots to the leaves (but not the fruits) of tomato plants even when virus was introduced below the soil surface eliminating the possibilities of aerial contamination (Oron *et al.* 1995). Our own data also show that internal contamination can occur but is probably of low level compared with external contamination (M.J. Carter, unpublished observations). However, a lower amount of virus inside a crop plant could potentially have an effect equivalent to a much higher external dose because it cannot be removed by either peeling or washing and may persist for longer because it is shielded from UV inactivation. Washing is not always efficient as a means of removing contamination, for instance HAV was found to adhere well to produce, especially lettuce (Croci *et al.* 2002). It is difficult to detect viruses in plant material and in only a few cases has contamination been shown directly, e.g. NoV in raspberries (Gaulin *et al.* 1999). Most associations are made by epidemiological connection and identification of the infectious agent in clinical samples from those affected (reviewed by Seymour and Appleton 2001).

Fruit and salad vegetables are traded around the world, originating from areas where sunlight permits growth but where water quality is not always assured. This trade either as raw materials or as ready-to-eat products can aid widespread virus dissemination. In 2003 an outbreak of hepatitis A occurred in the US, this affected several hundred persons in four states and resulted in four deaths. The source was eventually traced green onions imported from Mexico although virus was not identified in the food; identification was possible only by analysis of the chain of importation and transport (ProMED-mail 2003; 20031128.2946). In 1997 contaminated strawberries were used in a school lunch preparation that was distributed to 17 states in the US and may have exposed up to 9000 children (ProMED-mail 1997; 19970402.0689). A total of 200 cases

of HAV were reported in Michigan. Similarly, pre-prepared salads distributed across the US were responsible for a multi-state outbreak of NoV affecting over 300 persons (ProMED-mail 2000; 20000318.0377). Imported raspberries were responsible for an outbreak in Canada (Gaulin *et al.* 1999). Viruses are not destroyed by freezing or freeze-drying, although there are few studies of the latter process in contaminated food, in the laboratory freeze-drying is an efficient means of preserving virus infectivity and should be expected to preserve viruses in produce. Both hepatitis A (Reid and Robinson 1987) and NoV (Ponka *et al.* 1999) have been transmitted by frozen fruit. The growth in the worldwide trade in freeze-dried soft fruits (particularly raspberries and strawberries) offers another potential vehicle for transmission.

There are no data concerning levels or persistence of potential internal virus contamination of fruits and vegetables, although Sadovski *et al.* (1978) recovered poliovirus from cucumbers that had been irrigated with contaminated water 8 d earlier an upper limit to virus persistence was not established. In areas of high UV illumination surface contamination may be short-lived in the field, Badawy *et al.* (1990) studied virus survival on sewage irrigated grass in southern USA and found 2 log reductions in titre were achieved in just 8–10 h in the summer, slightly longer in the winter (16–24 h). However viruses can survive well on the surface of produce once harvested, a number of studies have indicated survival times of longer than a week and sometimes up to 30 d for a range of enteric viruses under typical storage conditions (Konowalchuk and Speirs 1974, 1975a,b; Badawy *et al.* 1985; Pirtle and Beran 1991; Kurdziel *et al.* 2001; Croci *et al.* 2002). Increasing temperature decreased virus survival times but conditions usually associated with produce storage were conducive to virus survival. It is not clear what effect microenvironments on a plant surface might have, waxy cuticles, hairy projections and curled, crinkled or convoluted leaves can all modify the way that virus contamination might behave, the amount of wetting will affect the way that a droplet dries whilst convoluted leaf surfaces could shield a virus from UV or increase local humidity. These effects are difficult to replicate experimentally. Data from Kurdziel *et al.* (2001) and Ward and Irving (1987) show that there is variability in the times that poliovirus may survive on different artificially contaminated plants stored at 4°C. Similarly these potentially sheltered environments suggest that washing processes will likewise vary in efficiency even over sections of the same plant.

These data suggest that contaminated fruit and vegetables might account for a large number of food-borne infections. A study in the UK indicated that these might present a significant opportunity for virus infections (O'Brien *et al.* 2000). However, the criticism frequently levelled at this implication is that these infections are simply not observed. To some extent this may arise from the assumption that community illness is entirely or mainly spread by the direct person–person route even though supporting data for this assumption is weak. When investigations have attempted to distinguish between person-to-person and food-borne illnesses in the community the latter can be identified by statistical means and suggest that there are potentially many thousands of such instances per year (see below).

8.3 Other foods

Few other foods are associated with primary contamination but a recent example is the consumption of raw liver from wild boar or venison. Both have resulted in the transmission of HEV to humans (Matsuda *et al.* 2003; Tei *et al.* 2003; Tamada *et al.* 2004). Consumption of raw liver is rare but virus contamination of utensils used in handling it should be borne in mind. Supermarket pork liver has also tested positive for HEV (Yazaki *et al.* 2003) – even in countries with no significant HEV infection. This suggests that pork liver is not a significant source of infection for humans.

8.4 Food handlers

Food handlers may contaminate foods at any point from harvest to serving. Toilet arrangements (including hand washing) should be provided for harvesters in the field to prevent contamination of produce as it is picked. Infected food handlers may or may not be symptomatic at the time of contamination. HAV infections may be mild or asymptomatic but large numbers of particles are nonetheless shed in the faeces. PCR technology has shown that even in symptomatic illness, NoV shedding may precede symptoms by 10–30 h and may continue (albeit it at a much lower level) for 1–2 weeks afterwards (Graham *et al.* 1994; Okhuysen *et al.* 1995; Estes and Leparc-Goffart 1999). NoV transmission by pre-, post- or asymptomatic food handlers has been demonstrated (Patterson *et al.* 1993; Parashar *et al.* 1998; Gaulin *et al.* 1999). Cowden *et al.* (1995) recommend a 48-h period of exclusion following the cessation of symptoms but as virus shedding is likely to continue for some weeks returnees must follow strict hand washing procedures. Inadequate hand washing/personal hygiene has been implicated in outbreaks of both NoV and HAV (Bean *et al.* 1990; Bidawid *et al.* 2004) and recently its effectiveness has been demonstrated. Bidawid *et al.* (2000b) considered the likely levels of hand contamination by HAV, and then estimated the resultant transfer of viruses to foods by handling. They concluded that up to 10% (or appprox. 1000 particles) could easily be transmitted via the fingers to food items (lettuce). If proper washing procedures were followed this transfer fell to 0·3–0·6%. Other experiments have shown that virus transfer rate is high immediately after

hand contamination but reduces as the virus dries (Larson 1985; Ansari *et al.* 1988; Springthorpe and Sattar 1998). Whilst this might imply that the window for contamination closes rapidly, it should be borne in mind that virus does survive drying onto the skin and reapplication of moisture (as in handling washed and wet foods) could then lead to increased transfers once more. Findings such as these emphasize the importance of proper hygiene in controlling food-borne infection from this source. However, given the likelihood that this will not be achieved in all cases, Koopmans and Duizer (2004) attempted to estimate the risks associated with food contaminated in this way. Using the value of 1000 particles for transfer (above), they reasoned that a 3 log reduction in virus viability would be required to ensure safety after such a contamination event. They then estimated the effects of various common food-processing procedures that might follow handling (e.g. freezing, fermentation, acidification, pasteurization, etc.). With the exception of boiling (and other high temperature treatments) no procedure achieved a 3 log reduction suggesting that viable virus would remain in almost all cases to present a threat to the consumer. Mariam and Cliver (2000) also showed that HAV would survive common pasteurization procedures for milk (30 min at 63°C and 15 s at 72°C).

The sudden onset of projectile vomiting and associated aerosolization of virus particles is particularly problematic when this occurs in a food handler. As vomiting may be the first overt sign of infection such a situation is virtually impossible to prevent. Given the survival characteristics of these viruses all uncovered food should be destroyed or cooked even if at some distance from the event. Decontamination of catering premises presents similar problems to that of hospitals. A sink into which an infected food handler had vomited was thoroughly cleansed with chlorine bleach. The following week the same sink was used to prepare salad and resulted in an outbreak of NoV amongst the consumers (Patterson *et al.* 1997). Food handlers certainly can contaminate foods but this is usually proved in only a minority of cases. Attempts were made to assign transmission as person-to-person or food-borne in an analysis of 2149 outbreaks occurring between 1992 and 1995 (ACMSF 1998). About 33% were attributed to NoV, 2% to RV and 0·5% each to astroviruses and small round (parvovirus)-like agents. Although not robust, this analysis suggested that many of the NoV outbreaks were food-borne and a food vehicle was suggested in 35 cases. Virus was detected in only two of the suspect foods (both oysters), in the remaining cases contamination was attributed to a food handler but subsequent investigation confirmed food handler infection in only 20%. Food vehicles implicated were diverse but included a variety of fresh fruits and salads as well as other items that would commonly either contain or be served with a salad or fresh fruit garnish. These included items such as sandwiches, pies, vegetable salads, gateaux, fish, lobsters and prawns. Consequently there was potential for primary (or at least remote secondary) contamination in 80% of these cases.

9. PERSON-TO-PERSON TRANSMISSION

Most enteric viruses are highly contagious, for most the infectious dose is believed to lie between 10 and 100 particles and for NoV possibly lower. Given rotavirus shedding at a level of 10^9 per ml, as little as 0·1 μl of stool contamination could contain an infectious dose. Such tiny quantities are invisible and easily retained on skin. Although hand washing is effective as a disinfectant (particularly alcohol-containing washes) in the case of rotavirus (Estes *et al.* 1979) infections are easily transmitted from person to person either directly or via contamination of objects and surfaces.

Vomit is also significant; 30 million particles may be shed by this route (Reid *et al.* 1988; Caul 1994) and RV may survive for up to 9 d in the air (Sattar *et al.* 1984). Proximity to vomit at the time of vomiting may be more significant than exposure later on; in a hotel NoV outbreak infection was related to distance between the secondary cases and the primary case at the time of the event (Marks *et al.* 2000) and in a hospital setting, persons in the proximity of a patient who vomited were at greater risk of acquiring NoV than those who actually cleaned up the vomit (Chadwick and McCann 1993). This implies that aerosolization is a potent means of infection of those in the immediate vicinity. Airborne transmission is distinct from respiratory transmission as the route of infection remains via the gut rather than the respiratory tract. Infection could result from the trapping of aerosolized droplets containing virus in the nasal passages and subsequent swallowing of virus. Alternatively aerosolized virus may travel some distance before settling and contaminating surfaces or uncovered food. Spread may be assisted by artificial ventilation (Chadwick *et al.* 1994).

Person-to-person transmission is clearly favoured by closed settings, persons cannot leave cruise-ships, hospitals or care homes and once a virus has been introduced by whatever route efficient spread within that setting is to be expected. In 2002 the CDC, Atlanta, recorded 21 outbreaks of NoV on 17 ships docking in the US (ProMED-mail 2002b; 20021212·6049); this included one vessel on which more than 200 cases were reported. Once a virus is present it can be very hard to eliminate even by thorough disinfection (Chadwick *et al.* 2000; Barker *et al.* 2004). Some ships showed serial outbreaks amongst each new set of passengers coming aboard. This is not surprising as even cleaning in a hospital context may fail – serial astrovirus infections were experienced in a bone marrow transplant unit despite vigorous attempts to disinfect the unit between patients

(Cubitt *et al.* 1999). Conventional cleaning of surfaces can fail to remove detectable NoV virus (Green *et al.* 1998; Barry-Murphy *et al.* 2000).

9.1 Person-to-person or food-borne?

Unsurprisingly, contact with an infected person is a major risk factor for contraction of these viruses: secondary attack rates of up to 95% have been reported for HAV within the home (Villarejos *et al.* 1982). HEV appears to infect less well in the domestic setting and secondary attack rates are lower (Purcell 1997) even though virus shedding may be more prolonged (Clayson *et al.* 1995b). Contact with an infected person was found to constitute the greatest single risk for infection by NoV in studies in the UK, US and the Netherlands (Mead *et al.* 1999; FSA 2000; De Wit *et al.* 2003). However this does not mean that all community spread must be via the person-to-person route, because unlike other viruses (which may be maintained entirely by this route), enteric viruses can also spread through food or water. It is difficult to estimate the contribution of each route to the total burden of infection in the community. Most figures consider only outbreaks, but the IID survey in the UK revealed the true extent of these 'missed' community infections for the first time (FSA 2000). This survey estimated that for every case of NoV that is laboratory confirmed, over 1500 occur in the community. The report attributes community cases (almost) entirely to person-to-person transmission but presents no data on this point. Recently De Wit *et al.* (2003) have made a thorough attempt to determine the extent of food-borne transmission in the community. They found that person-to-person transmission was indeed common, much of it occurring in the home and in many cases actually involving contamination of food prepared for others. This is not counted as 'food-borne' infection (see above). The analysis also showed for the first time that person-to-person transmission alone could not account for all community illness and the authors concluded that 12–16% of infections could be attributed to contaminated food/water entering the household. In the UK there are some 9·5 million cases of IID annually and has been estimated that some 16% ($1{\cdot}52 \times 10^6$) are caused by NoV. Assuming that all of these took place in a household of four persons and that all persons in each household were infected, this would correspond to a minimum of 380 000 infected households. Lastly, if 12–16% of these resulted from incoming contaminated food as above, then contaminated food could instigate directly some 45–60 000 food-borne cases in the UK per year as a minimum estimate. Estimates in the Netherlands using less conservative assumptions suggest 80 000 food-borne NoV infections could occur annually even in this smaller country (De Wit *et al.* 2003).

10. VIRUS DIAGNOSIS AND DETECTION

10.1 Clinical samples

An assessment of the impact of these viruses in human disease requires the provision of a sensitive and reliable detection method for application to clinical samples. Routine reagents for the detection of rotavirus, HAV and poliovirus have been available for some time but technology for the other viruses of interest has only recently moved from the specialist laboratory into widespread use. As a result the burden of disease contributed by other gut-infecting viruses has not been so clearly established. Diagnostic reagents have formerly been hard to provide and in their absence perhaps the simplest method has been to examine the faeces using electron microscopy. This is a catch-all method and requires no prior knowledge of the virus in order to detect it but it is expensive, makes high operator demands and its efficiency depends on a virus being readily recognizable; Viruses that are large, of distinctive appearance, and present in significant numbers ($>10^6$ particles per ml) are relatively easily observed. However, small, diffuse or fuzzy viruses can often be overlooked. Identification makes high demands on the operator and this is even worse when a virus may have several distinct appearances (see astroviruses). Historically it is thought that this method has identified AdV and rotavirus infections quite well but has been less successful in the cases of caliciviruses, astroviruses and parvoviruses.

Most gut-dwelling bacteria are routinely identified by culture; however, this route has not been open to the detection of viruses. All viruses require living cells as hosts and are totally dependent on the processes that their host cells are able to provide, e.g. for protein synthesis and protein processing, but the gut is a very specialized habitat in virological terms. It contains a multiplicity of different cell types, each having a different enzyme content and surface protein composition; they may even vary in these properties at different stages of their differentiation. Furthermore, they are all bathed in a solution of the various secreted products produced by other specialized cells, notably of course proteases and bile salts. These features make the gut a very difficult cellular environment to mimic in culture. A virus may replicate in one type of cell, at one stage of its differentiation and may require soluble products released from quite different cells entirely. It is probably for this reason that gut-replicating viruses have been very difficult to culture in the laboratory. Those that can be cultivated often require that the culture be supplemented with proteases (usually trypsin) and in one case (porcine enteric calicivirus) with duodenal juice, bile salts being the active ingredient (Chang *et al.* 2004). Specialized cells are often needed, e.g. differentiating colonic carcinoma cells for astroviruses. Viruses that penetrate beyond the gut, invading

other tissues are often (but by no means always) simpler to cultivate. The significance of this point cannot be overstated, most extracellular parasites will grow reasonably efficiently in a broth which mimics the gut contents, but this is not true of viruses and there are very good reasons why the characterization and detection of the viruses of gastroenteritis have been problematic. Culture for viruses is not generally used even to identify agents present in a faecal sample. The chances of using such a procedure for the detection of the lower quantities of virus present in food are still more remote.

In view of the above difficulties in EM detection, reagent availability in the past and difficulties in culture most workers have acknowledged the existence of a 'diagnostic gap' represented by the large proportion of infections for which no obvious cause could be found. Even in the IID survey in England and Wales, when causative agents were vigorously pursued, target organisms were not identified in 63% of community samples and 45% of General Practice samples (FSA 2000). Given the difficulties in virus diagnosis, it was always likely that a large part of this diagnostic gap would probably be caused by viruses, but probably by the smaller, less well-characterized and less-readily observed viruses. The extent of any such bias in detection has recently become clearer with the development of objective and sensitive methods for the detection of many viruses: the development of recombinant antigens for astroviruses and especially NoV, and the development of ELISA systems for both have at last provided a reproducible supply of antigen from which to prepare and validate diagnostic serological reagents as well as to conduct serosurveys of the incidence and age-at-first-infection profiles of these viruses. This type of detection method has been used in the IID survey in England and Wales – although not all tests in all cases (FSA 2000), and in similar studies in Europe (Koopmans *et al.* 2000) and the US (Mead *et al.* 1999). These studies all support the conclusion that virus-associated enteric illness has been greatly underestimated and currently suggest (in the absence of concrete data) that most, if not this entire diagnostic void is made up of NoV infections. Similarly data from the FSA survey in England (2000) suggested that for every case of NoV actually detected in the diagnostic laboratory, on average 1562 have probably occurred in the community.

10.2 Food samples

Virus detection in food and water (i.e. preinfection) is even more problematic than detection postinfection, in clinical samples. Levels of virus are orders of magnitude lower and the challenge is to detect a potentially infectious dose (perhaps as low as 10 particles) in a quantity of a size likely to be consumed as a single portion. As these viruses can either not be cultivated or cultivated only with difficulty, most detection methods have concentrated on PCR technology. This has proved extremely successful when applied to NoV detection in water and shellfish and can even provide quantitative information (Laverick *et al.* 2004; Le Cann *et al.* 2004). However PCR-based detection has two problems, the first is that most of these viruses are RNA viruses, PCR cannot be used unless the RNA has been reverse transcribed (RT) to manufacture a DNA copy and the efficiency of this RT step is notoriously variable. Secondly PCR targets only a short section of the virus genome (usually 200–300 bases) and reveals nothing at all about the presence or integrity of the rest of the genome. Strictly then, even quantitative PCR for such a virus detects only the levels of cDNA to a specific region of the virus, it cannot tell us how the numbers of DNA copies detected relate to the number of RNA copies actually present in each case and nor can it tell us whether the target detected was present as an intact genome inside a viable particle or present as a short section of free RNA released by virus degradation. The first of these difficulties can be tackled by careful incorporation of internal controls, although these may of themselves interfere with target amplification. The second is harder to address. It is often assumed that RNA degradation occurs in two phases; virtually none at all whilst the particle is intact followed by rapid and total degradation once the particle is breached. Consequently levels of free RNA should be insignificant and most detectable RNA would be contained inside an intact particle. However it is not clear that this assumption is valid; whilst Slomka and Appleton (1998) used FCV as a model for NoV and found that RNA was indeed rapidly degraded after inactivation of the virus in shellfish they also confirmed that some PCR-positive samples were not cultivable. Similar findings were reported for poliovirus and bacteriophage MS2 (Shin and Sobsey 1998; Sobsey *et al.* 1999). Recently a study by Nuanualsuwan and Cliver (2003) suggests that the situation could be yet more complex. These workers inactivated calicivirus and picornaviruses (including hepatitis A) by exposure to high temperature (72°C), UV radiation and hypochlorite disinfection. They found that infectivity of all viruses could be vastly reduced by these means. However, the RNA inside the particle appeared to survive, the reduction in infectivity being attributed to a loss of capsid function. Inactivated particles could no longer attach to host cells and were no longer recognized by antibodies. Despite this failure the RNA remained intact within them and continued to be detected by PCR; furthermore it was still protected from degradation by hydrolytic enzymes suggesting that the disjunction between PCR detection and virus viability would become worse with time. These findings are of great significance as they suggest that PCR-based detection and quantitation would also detect virus particles

that are inactive and pose no threat to a consumer. This would overestimate the extent of virus contamination. Antibody-based detection methods (or PCR detection following an antibody-mediated concentration step) should overcome this effect and this has also been observed (Sobsey *et al.* 1999). A technical account of the primers and conditions required for virus detection is beyond the scope of this review but have been summarized elsewhere (Sair *et al.* 2002).

One drawback of PCR-based detection is that the test consists of the observation of amplification or failure to amplify. Specificity relies on the choice of primers which should amplify only the selected target viruses. This approach is intrinsically unsuitable for the identification of multiple viruses simultaneously. It yields no information on the presence of other virus types not specifically sought by the procedure, or more distantly related to the 'known' viruses from which primers were designed. PCR for NoV for instance can identify only 90–95% of EM-positive samples, presumably because of inherent sequence variability within the virus family. However microarray technology now offers the ability to use multiple and redundant PCR primers to target and amplify a range of viruses present in a sample. Specificity is then achieved at the array hybridization stage when hybridization to a microarray plate containing several hundred probes for individual viruses or groups of viruses detects each simultaneously. Such detection technologies offer the chance of defining at last the true extent of virus contamination of both food and the environment.

11. ALTERNATIVE INDICATORS OF VIRUS CONTAMINATION

In view of the difficulties in detecting viruses above, much use has been made of indicator organisms to provide clues to the likelihood of virus contamination (reviewed by Kator and Rhodes 1987). Cultivable enteroviruses such as poliovirus have been extensively used. This agent has been ubiquitous in sewage-polluted environments because of widespread vaccination in the community and the resultant faecal shedding from vaccinees. However it is difficult and expensive to routinely extract and assay poliovirus from contaminated food and water, further the WHO aim to eliminate this virus from the world and work with it is already subject to control. As a substitute many workers now suggest AdV (see above). However an alternative approach has been to use nonhuman viruses such as the bacteriophages (Power and Collins 1989, 1990) and chief amongst these are the male-specific bacteriophages of the Levivirus family such as MS2. These bacteriophages contain an RNA genome of similar size to the picorna-, calici- and astroviruses and have particle sizes in a similar range. Bacteriophage are frequently found in faecally contaminated materials (and shellfish associated with gastroenteritis outbreaks) and are thought to show similar stabilities to the enteric viruses. These data suggest that they may be good models for virus contamination in these systems (Dore *et al.* 1998, 2000). However it should be borne in mind that indicator organisms such as these can only indicate the possibility of human virus contamination; they cannot prove either its presence or its absence. F+-specific bacteriophage can be specifically enumerated using an engineered pilus-bearing *Salmonella typhimurium* host that cannot be infected by non-RNA coliphage (Havelaar and Hogeboom 1983). Use of this host yields data relatively free of contaminating somatic coliphage but does not easily separate contamination of animal origin from that of human. Further work would be necessary to make such a distinction using oligonucleotide hybridization (Beekwilder *et al.* 1995; Hsu *et al.* 1995). At present there are no data concerning the use of these organisms as potential indicators of faecal contamination for soft fruits and vegetables. Mariam and Cliver (2000) attempted to use bacteriophage to determine the extent of virus inactivation likely in response to certain treatments in various food processes and Dawson *et al.* (2005) used phage to monitor the survival of viruses on soft fruits. Slomka and Appleton (1998) used feline calicivirus in a similar manner. However these experiments require careful analysis as neither are exactly equivalent to the viruses of interest. Mariam and Cliver (2000) found that both MS2 and ΦX174 were more labile than HAV in their analysis. However they nevertheless concluded that these organisms could be useful if appropriate relationships could be determined between phage inactivation rates and those of viruses of concern. Such work demands parallel experiments using the actual viruses of concern to 'calibrate' the response of the indicators in a variety of circumstances and such data is urgently required.

12. CONCLUSIONS

Food-borne viruses are ubiquitous; it is likely that their incidence has been underestimated in the past not only in terms of the number of infections but also in terms of the frequency with which they are food-borne. Sewage treatment without disinfection should be recognized as inadequate and UV treatments of final effluents should be encouraged to protect both recreational waters and especially shellfish harvesting areas. Intrinsic contamination of foods other than shellfish needs to be considered and more work is needed to characterize the uptake, survival and removal properties of viruses in plant tissues. Given the difficulty of excluding (or even identifying) food handlers likely to be shedding virus at any one time and the growth in national/international trade in foods requiring minimal cooking we may expect food-borne viruses to increase in significance in the future. This effect may be of most significance in the case of HAV where the divergence in

infection patterns between producer and consumer countries is greatest.

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