CHAPTER 8

Microbiological Hazards and Their Control: Viruses

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INTRODUCTION

Viruses differ significantly from bacterial pathogens because they are obligate intracellular parasites and can replicate only within an appropriate living host cell. The source of all viruses is thus a previously infected individual who sheds infectious particles into his or her immediate environment. Transmission to another host can be direct, as in person-to-person spread (e.g., through aerosols created by a sneeze or vomiting), or indirect, involving some other agent as a carrier for the virus. Food-borne transmission is of the indirect type. All viruses that infect via the enteric tract (and are shed in feces) are potentially capable of food-borne transmission. Direct person-to-person spread can also occur if the opportunity arises; direct feces-to-mouth transmission is common in children, and airborne spread, leading to contamination of distant surfaces, has been reported.

Foods are contaminated with viruses as a result of the distribution of fecal- or vomitus-derived viruses through the environment, eventually contaminating the food or water of another potential host. This process is highly variable. It could, for instance, result fairly directly from an infected food handler contaminating food immediately before consumption, or it could be the end result of a prolonged distribution process moving virus from fecal material to river and estuary and, perhaps eventually, to seafood. Generally, these indirect routes require more time than direct spread, and the stability of the virus particle will be key to its successful distribution. Evolution has ensured that viruses of this type are especially fitted for survival in transit, which has a direct influence on their ability to survive in processed food.

Water, either ingested directly or used as an ingredient or washing agent during food processing, is the chief vehicle for disseminating enteric viruses. It is also significant in carrying these viruses to plants in the field (e.g., in irrigation water or sprays), and to the most significant vector of food-borne viral illness, molluscan shellfish. Most food-borne viruses survive well in water. Their survival is assisted by high protein content and high ionic strength, particularly calcium and magnesium ions, which tend to stabilize the particles. These conditions are often found in sewage and sewage-contaminated water. The adsorption of virus to suspended solids such as clays or organic matter can also protect the particles from inactivation.

Viruses contaminating food are there as a result of a dilution and distribution process that may have taken a significant time. Because viruses have an absolute requirement for living cells in which to replicate, they cannot increase in numbers during this distribution phase or within contaminated food during storage. In fact, the amount of contaminating viruses may decrease during storage; this can be assisted by

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treatment with heat, irradiation, chemicals, or other factors such as the pH changes associated with the fermentation of foodstuffs. Thus, the levels of active viruses in foods must be expected to be generally very low. Consequently, and with very few exceptions (mainly molluscan shellfish), viruses are only rarely detected in foodstuffs before consumption. In fact, it is only molluscan shellfish that are routinely monitored for the presence of viruses; in all other cases, a contaminating virus is usually inferred only retrospectively once consumption has led to illness. Direct contamination of food from an infected food handler could deliver a more concentrated virus loading to a foodstuff, but because this could occur very sporadically, at any stage up to and including serving, testing food for virus contamination from this source is generally not a viable option.

There are problems in assessing the importance of viral food-borne illness. Figures generally suggest that it is relatively low in adults, with the exception of the caliciviruses. However, there is an almost complete lack of data addressing endemic levels of infection. Official figures refer almost entirely to "outbreaks" of food-borne virus illness, that is, to epidemic behavior. Such outbreaks could result from the simultaneous consumption of contaminated food by many people, or equally from the infection of a single person via contaminated food, followed by more direct person-to-person spread within the person's immediate surroundings. It is probably significant that many outbreaks of infection are observed in closed communities where hygiene may not be advanced and where there is increased potential for person-to-person spread, such as in childcare centers or nursing homes. It is this subsequent transmission that renders the original food-borne infection statistically "visible." Food-borne infections such as these would not be recorded if, for instance, they had occurred in a private home and affected one or only a few relatively fit persons. Consequently, the figures available probably represent only the tip of the iceberg as far as the incidence of food-borne virus transmission is concerned. Some measure of the extent of underreporting was gained from the Infectious Intestinal Disease Study conducted in England during the period 1993–1996.[48] This study concluded that underreporting was most severe for diarrhea-causing viruses, and that for every officially reported case of infection with Norwalk-like virus (NLV), there were actually 1,562 cases in the community at large.

FOOD-BORNE VIRUSES

General Features

There are two broad classes of virus that may infect through the gut. The first class uses the enteric tract as the portal of entry to the body, but subsequently spreads elsewhere around the body. Examples of this type of infection would be spread to muscle tissue of the skeleton or heart (as in the Coxsackie B viruses), to the meninges and central nervous system (polio and occasionally other enteroviruses), or to the liver (hepatitis A and E viruses). Viruses in the second class are true inhabitants of the gut. They replicate significantly only in this tissue and induce signs and symptoms of a typical "gastroenteritis." This is mainly diarrhea, but can be accompanied by differing degrees of vomiting. Viruses like this include the caliciviruses, rotaviruses, adenoviruses, and astroviruses. Finally, there are a few agents that are detected routinely in the gut, such as the pico-birna viruses. These are poorly characterized and do not seem to be associated with disease. The general features of these viruses, as well as their size, structure, and genome content, are provided in Table 8–1.

Difficulties in Culture and Diagnosis

The gut is a very specialized habitat in virological terms; 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). There is a multiplicity of different cell types in the gut, each with a different role. This is reflected in a differing complement of enzymes and surface proteins; cells may even vary in these properties

Table 8–1 Food–Borne Viruses

Virus	*Members*	*Features*	*Associated Illness*
Caliciviridae	Norwalk-like and Sapporo-like viruses	34 nm nonenveloped particles with distinctive morphology (surface cups). Single stranded +ve stranded RNA genome.	Norwalk-like: explosive projectile vomiting in older children/young adults. Noncultivable. Sapporo-like: more common in younger children believed to be milder in effect, noncultivable.
Reoviridae	Rotavirus groups A–E; only groups A–C are found in man. Group C is rare and group B is uncommon except in China.	Multi-layered, nonenveloped 70 nm particles, enclosing double-stranded RNA genome. Only type A is cultivable and requires a trypsin supplement.	Diarrhea common in the young, decreasing in frequency with age. Illness returns in the elderly.
Adenoviridae	Adenovirus group F, types 40 and 41	100 nm, nonenveloped icosahedral particles, ds DNA genome. Cultivable in some cells (e.g., Graham 293)	Mild diarrhea, may be prolonged virus shedding. Mainly affects children.
Astroviridae	Types 1–8	28 nm nonenveloped, star motif may be visible. Single-stranded +ve RNA. Appearance can be variable; may be mistaken for calici or parvoviruses. Cultivable in differentiating colonic cells with trypsin supplement.	Mostly infects children, but higher serotypes are rarer and can cause significant disease in adults. Relatively mild, but probably underestimated.
Picornaviridae	(Enterovirus) Poliovirus Coxsackievirus ECHO virus Enterovirus	28 nm nonenveloped particles, single-stranded, +ve sense RNA. Grow relatively easily in a variety of human and primate cell lines.	Mainly asymptomatic, can induce muscle pains (Bornholm Disease), cardiomyopathy, meningitis, and CNS motor paralysis (most common with polio).
	(Hepatovirus) Hepatitis A virus	As above, cultivable with difficulty.	Hepatitis, mild in the young.

continues

Table 8–1 continued

Virus	*Members*	*Features*	*Associated Illness*
Unclassified	Hepatitis E virus	Calicivirus-like particle structure, containing single stranded +ve RNA of unique genomic organization. Noncultivable.	Similar features to HAV but more severe. Often fatal if contracted in late pregnancy.
Parvoviridae	Wollan, Ditchling. Cockle agents	Smooth featureless 25 nm particles. Single-stranded DNA genome. Poorly characterized, noncultivable.	Widespread shellfish-associated outbreaks, largely controlled through cooking.
Coronaviridae	Uncharacterized	Large enveloped virus, fragile, noncultivable. Food-borne transmission unlikely in view of their fragility.	Associated with neonatal necrotising enterocolitis.
Toroviridae	Uncharacterized	Fragile, enveloped virus, may resemble Berne virus in horses. Noncultivable, food-borne transmission unlikely as above.	Unknown

at different stages during their differentiation. Furthermore, all gut cells are bathed in a solution of the various products that are secreted by other specialized gut cells, notably, of course, proteases. These features make the gut a very difficult cellular environment to mimic in culture. For instance, a virus might replicate in one particular type of cell at one stage of its differentiation and could also require extracellular soluble products released from quite different cells entirely. For this reason, viruses that replicate in the gut have been very difficult to culture in the laboratory, and some still cannot be grown. Those that can be cultivated often require that the culture is supplemented with proteases, usually trypsin,[5] or even with duodenal juice.[37] Specialized cells are also sometimes needed (e.g., differentiating colonic carcinoma cells for astroviruses).[49] Viruses that penetrate beyond the gut and invade other tissues are generally (but by no means always) simpler to cultivate than those that reside in the gut.

This difficulty in cultivation has two important effects. First, virus detection can be problematic. Usually, a cause of infectious gut illness can only be established in approximately half of the cases that are investigated. It is likely that a large proportion of those cases of infection that remain unidentified will in fact be found eventually to be caused by viruses, but by viruses that hitherto have been missed because they are not culturable, and because there is no adequate diagnostic test for this particular virus. The only catch-all method is electron microscopy, but this depends on a virus being readily recognizable by its shape and present in large numbers (many workers quote a minimum requirement of 10^6 particles per ml before observation becomes

even reasonably likely). Large viruses like the rota- and adenoviruses are distinctive under the microscope, but small, diffuse, or fuzzy viruses may often be overlooked, and their identification makes high demands on the operator. Other tests may be less demanding in use, but require characterized reagents such as antibodies for agglutination or ELISA-based methods and primers for PCR-based detection. Thus, these tests can only find viruses that are already characterized and known; they cannot discover new ones. These shortcomings bias the relative detection frequencies of viruses associated with enteric infection. Although the extent of any such bias is not clear, it is difficult to avoid the conclusion that virus-associated enteric illness is likely to be substantially underestimated, and that this problem will be worse when considering the smaller, less distinct viral agents.

Second, if it is not possible to culture a virus *in vitro*, then that virus can only be investigated *in vivo*. If the only hosts are humans, such experiments become expensive and cumbersome, if not ethically unacceptable. For this reason, many of the fundamental investigations into stability, transmission, and reinfection that are required have not been done or have not been confirmed.

POTENTIALLY FOOD-BORNE VIRUSES

Table 8–1 summarizes the main viruses associated with enteric infection. Several of these are as yet uncharacterized and of necessity will not be considered in this chapter. Any virus that is enterically transmitted is potentially transmissible via food, although person-to-person spread may be more common. This is especially true of viruses that affect children and infants. Most enteric viruses fall into this category. Infection is acquired early in life and becomes less common through late childhood, adolescence, and adult life, increasing in significance once more in the elderly. The significance of food-borne infection can thus depend on the types of food that are usually ingested by children. Chief among infections of adults are the Norwalk-like viruses (NLVs, previously called the small round structured viruses–SRSVs), and the Sapporo-like viruses (SLVs), two related members of a virus family called the *Caliciviridae*. Viruses of the NLV type are the most common viral cause of explosive outbreaks of projectile vomiting in adults. In recent years, reports of viral gastroenteritis, particularly NLV outbreaks, have shown a dramatic increase in England and Wales; 418 cases were identified in 1992 and 2,387 in 1996.[4] In 1994, reports of NLV outnumbered those of *Salmonella* for the first time. This is probably due in part to increased awareness and referral for diagnosis, but it indicates the extent to which such infections may have been missed in the past, and probably continue to be missed today.

Increased surveillance for viral gastroenteritis was introduced in the United Kingdom between 1992–1995, and this has yielded some very interesting results. A preliminary data set was obtained for 2,149 of 2,680 outbreaks during this period. Of these cases, 25% were attributed to *Salmonella*, 33% to NLV, 2% to rotaviruses, and 0.5% each associated with astroviruses and small round (parvovirus) like agents. Twenty percent of cases were of unknown etiology, and approximately half of these were believed due to viruses. The features of all of these viruses associated with food-borne transmission are described briefly in the following sections.

Caliciviruses (NLVs and SLVs)

NLVs and SLVs have different appearances under the electron microscope, and the relationship between them has been debated for some time. SLVs have an obvious and unique structure under the electron microscope; they appear to be covered in cup-like depressions, from which the virus takes its name (calici = a cup). However, the NLVs appear fuzzy. Structural studies using cryo electron microscopy have now revealed considerable underlying similarity in structure, and molecular studies have reinforced these. It is now clear that these two agents are related.[11]

NLVs

NLVs were first identified following an outbreak of enteric illness among adults in the town

of Norwalk, Ohio that has now given its name to all viruses of this type. NLVs are associated with sporadic outbreaks of diarrhea and vomiting among young adults and older individuals. The disease is more common in winter than at other times of the year, and had been tentatively named winter vomiting disease in 1940, although its cause was not known at that time. NLV is characterized by an incubation period of up to 48 hours, with illness lasting for 1–2 days. This virus differs from most other agents of viral gastroenteritis in that it mainly attacks adults and it frequently induces a high level of vomiting among infected individuals. In fact, sudden, explosive, projectile vomiting may be the first obvious symptom of infection. For this reason, many cases of NLV are actually identified at work. Thus, the implications if a food handler should be infected are obvious.

In addition, although there are multiple serotypes of NLV (probably more than eight), immunity to each seems to be short lived. Thus, individuals may be protected for only a short period of time (months) following an infection before they become susceptible to the same virus again.[35] Some people appear to have an inherent resistance to infection; these individuals remain symptom free, even in the absence of antibody. However, sensitive persons may require several bouts of infection by the same virus before antibody levels are sufficient for some protection. Even in middle age, only approximately 50% of people are seropositive. Although NLV does cause outbreaks in institutionalized settings where person-to-person spread can be significant, it is also associated with multiple simultaneous exposure events where otherwise healthy adults consume virus-contaminated food. Such outbreaks have minimal person-to-person transmission components, and 7–8% of outbreaks in the United Kingdom are described as mainly or chiefly food-borne in nature. However, this attribution is not particularly robust; the real figure could be significantly higher.

NLV is spread chiefly by water. Significant outbreaks of NLV have been associated with the commercial manufacture of ice from contaminated wells, soft fruits, and fruit juices. However, chief among the sources of NLV outbreaks is the consumption of raw or partially cooked shellfish. Molluscan shellfish are filter feeders, often farmed or growing naturally in estuarine locations. In Europe, most estuaries are contaminated with sewage effluent, and any viruses that are shed in the water from communities living upstream become concentrated within the bodies of the shellfish. Frequent outbreaks of gastroenteritis have been associated with fecal bacteria that was also concentrated in this way, but simply relaying the shellfish in clean water (depuration) is often sufficient to allow them to purge themselves of such bacteria. Unfortunately, this process is inefficient in ensuring the removal of viruses. Current European Union law regulates the sale of shellfish according to the levels of coliform bacteria they may contain, though this is no guarantee of virological cleanliness. Consequently, each year, a number of outbreaks are attributed to the consumption of raw shellfish, particularly oysters. Outbreaks show a slight seasonality, for instance, shellfish seem to clear viruses less efficiently in the winter when sea temperatures are lower, and outbreaks may also increase at such times. Eating habits can also be a contributory factor. For example, in the United States, there are often outbreaks around February 14th and around Thanksgiving because oysters are traditionally eaten there at these times.

SLVs

SLVs cause illness more commonly in children and account for some 3% of hospital admissions for diarrhea in both the United Kingdom and the United States. Most children are seropositive by age 12 and seem to become infected between three months and six years of age. The disease is particularly common in institutionalized settings such as schools and daycare centers. Incubation is between 24–48 hours, and illness is usually mild and short lived, with diarrhea tending to predominate. Any clinical differences reported in the illness induced by SLV and NLV seem to reflect more the age of the patient than an intrinsic property of the virus itself. On those occasions where SLV may cause illness in adults, the signs and symptoms are indistinguishable from those of NLV.

An assessment of the stability of NLV is particularly difficult because the virus can only be cultivated in volunteers. However, despite this limitation, a few valuable studies have addressed this point, and it is worth commenting on the sensitivity of NLV to chlorine used in the treatment of potable water. Reports from the United States show that the virus resists treatment at a peak level of 5 mg/l for 30 minutes. Thus, NLV can survive water chlorination to this level and has even been detected in tap water in the United Kingdom (although it is not known if it was infectious at that point). NLV is inactivated at levels of 10 mg/l, and this is used to decontaminate water supplies if contamination is suspected. The virus is relatively more chlorine resistant than polio and human rotaviruses (rotavirus is inactivated at levels greater than 3.5 mg/l).[26] NLV is acid stable, surviving at pH values as low as 2.7 for three hours at room temperature, and also relatively heat stable (resists 60 °C for 30 minutes).[13] Because the virus cannot be grown, feline calicivirus (FCV) is frequently used as a model.[44] Although it is true that the feline virus will probably mimic the behavior of NLV in terms of where it tends to accumulate, it is a relatively poor model for stability. For example, FCV is considerably more acid labile than NLV. Like all other enterically transmitted viruses, NLV lacks an envelope and thus tends to resist lipid solvents that would otherwise inactivate viruses by stripping their lipid envelopes. For instance, NLV is not inactivated by 20% ether at 4 °C for 18 hours.[13]

Rotaviruses

Rotaviruses are members of the family Reoviridae (Table 8–1) and account for some 3.5 million cases of diarrhea in the United States each year. This equates to 35% of hospital admissions for diarrhea each year. Even in a developed country like the United States, approximately 120 children die each year from this virus, and fatalities are probably far more numerous in less developed countries. The peak age for illness is between six months and two years; by four years of age, most people have been infected.[27] Immunity to rotaviruses is long lasting and secretory immunoglobulin A (IgA) in the gut plays an important role.

Group A rotaviruses exist in nine serotypes, and variations in other proteins increase the antigenic diversity of this group. However, as time progresses, individuals are exposed to many different variants of rotavirus, and immunity gradually accumulates. Thus, frequency of illness decreases with age. However, silent secondary re-infections can occur throughout life (as in parents caring for infants), thereby providing another means for the virus to spread in the community. Illness usually develops after an incubation period of four to seven days and presents as diarrhea and vomiting lasting approximately one week. In the United Kingdom, only some 2% of outbreaks were attributed as food-borne. The viruses are stabilized by calcium ions, which promote integrity of the particle.[42] Ionic detergents can reduce infectivity (e.g., 0.1% sodium dodecyl sulphate, or SDS), but non-ionic detergents can actually increase virus titre by breaking up aggregates and dispersing the particles. Viruses are very resistant to acid conditions and are stable over a wide pH range from 3.0 to 9.0.[14] They can retain infectivity for months at 20 °C[42] and also resist heating to 50 °C. Rotaviruses tend to survive desiccation well and are reduced in titre by less than 10-fold during drying (A. Bosch, personal communication). Once dried, the virus survives well on either porous or nonporous surfaces, being relatively unaffected by temperature or relative humidity. Infectivity of dried viruses reduces in titre by just more than 2 logs over a 60-day period, regardless of substrate, temperature, relative humidity, or the presence of accompanying fecal material. This makes rotavirus one of the most stable of enteric viruses, second only to hepatitis A virus in this respect.[1]

Adenoviruses

There are 47 serotypes of adenoviruses, but only types 40 and 41 cause enteric illness. Adenoviruses account for some 5–20% of U.S. hospital admissions for diarrhea, mainly in children below two years of age.[27] Incubation lasts

three to ten days, and illness (watery diarrhea) may last for one week. However, prolonged shedding of the virus has been reported. As children age, exposure to adenovirus infection gradually increases the levels of immunity. Only 20% of children below six months of age have antibody to these viruses, but by age three, this has risen to 50%.[27] In the United Kingdom, adenovirus-related outbreaks are extremely rare; only one case was identified between 1992–1995, and this was not believed to be food-borne.[4] Clearly, this is in stark contrast to the antibody prevalence figures and implies that infection may be mainly in the community and doesn't follow outbreak behavior.

There are few studies that have specifically examined the stability properties of the enteric adenoviruses, but most workers agree that the viruses are certainly stable between pH 5 and 9 and up to 45 °C.[17] Infectivity is rapidly (10 minutes) lost above 56 °C, and this is associated with particle disintegration.[40] Other workers have examined the stability of the enteric adenovirus type 40, when dried on to fomites.[1] Adenovirus is one of the least stable of the enteric viruses under these conditions, and its behavior seems to resemble that of poliovirus rather than rota-, astro-, or hepatitis A viruses, which tend to be more robust. Desiccation itself causes a 100–1000-fold decrease in titre. However, infectivity persists for seven days on nonporous substrates, it is stabilized by the presence of accompanying fecal material, and it is unaffected by levels of relative humidity. Adenoviruses survive less well on porous surfaces, and the accompanying material seems to decrease its stability. Survival is better at 4 °C than at 20 °C on either surface. In liquids, adenovirus stability closely resembles that of the astroviruses, which are small, round RNA containing viruses responsible for enteric infections mainly in the young of animals and humans. Adenoviruses survive relatively well in tap water; log titre reduction values of 3.2 were observed after 60 days at 20 °C. The virus is also disinfected by free chlorine, giving log titre reduction values of 2.5 and 3 after two hours in the presence of 0.5 and 1 mg/l free chlorine, respectively. Again, this behavior mimics that of human astroviruses.[2,3]

Astroviruses

Astroviruses (serotypes 1–8) account for some 5% of hospital admissions in the United States, almost entirely of children.[27] By seven years of age, 50% of children are already seropositive for the most common serotype (type 1), and this reaches 75% by age 10. Illness is generally mild and lasts some two to three days after an incubation period of similar length. This has led many researchers to dismiss these viruses as causative agents of significant disease in humans. However, this is not necessarily true, first because the higher serotypes are less common, thus infection is delayed and occurs among older children and adults. Preexisting antibody to other serotypes may modify the severity of any resulting illness but may not prevent the occurrence of clinical disease. In Japan in 1995, 1,500 older children and their teachers were affected in a widespread food-borne outbreak caused by astrovirus type 4,[33] and symptomatic illness has also been seen in adults in the United Kingdom and France. Second, astrovirus diagnosis can present particular problems that may lead to an underestimation of the number of cases. Diagnosis still largely relies on electron microscopy, but particle morphology alone is not necessarily a good guide. Astroviruses may frequently be mistaken for small round (parvovirus-like) agents and even for NLVs.[23] This is a significant finding, because a recent survey in the United Kingdom identified only nine outbreaks associated with astrovirus (and a further two mixed infections that also contained NLV), of which approximately 20% were thought to be food-borne.[4] A further four outbreaks of illness were associated with small round virus, an agent usually assumed to be parvovirus and routinely dismissed as the causative agent of the outbreak. However, half of these cases were believed to be food-borne, and in view of the potential for misidentification, these agents require further investigation. Should these cases actually represent misidentified astroviruses, then the proportion of astrovirus outbreaks classified as food-borne could be as high as 30–45%. Recently, an ELISA-based detection kit has been produced,

which could help to answer these questions if it is widely adopted.

Astroviruses survive desiccation well, dropping by only 10-fold with or without accompanying organic material. If the virus is dried on to a nonporous surface, accompanying fecal matter can boost relative survival by 10–100-fold, with infectivity persisting for up to 65 days and probably much longer at 4 °C. Astrovirus survival is greatly reduced at increased temperatures and can decay completely within 10 days at 20 °C on a nonporous surface. Adenoviruses survive equally well on porous surfaces at 4 °C regardless of the presence or absence of fecal material, infectivity persisting for more than 90 days. This suggests that environmental temperature may play an important role in the seasonality of astrovirus outbreaks because indirect transmission would be assisted by decreased temperature (A. Bosch, personal communication).

Astrovirus survives well in dechlorinated (tap) water[3] and reduces in titre by 100-fold after 60 days at 4 °C. This is increased to a 3.2 log reduction after 60 days at 20 °C, which is a very similar stability to that observed for human rotavirus and adenovirus type 40, where the same reduction was seen at 20 °C. Free chlorine seems more effective at disinfecting astrovirus-contaminated water than water contaminated with hepatitis A or human rotavirus. Astrovirus titres were reduced by 2.5 logs after one hour at a free chlorine concentration of 0.5 mg/l. This increased to 3 logs in the presence of 1 mg/l free chlorine. However, in both cases, residual infectivity was still detected after two hours of treatment when the log titre reduction was 4.17.[3]

Hepatitis Viruses

Hepatitis viruses A and E are classified differently. Hepatitis A virus (HAV) is the only member of a single virus genus (*Hepatovirus*) of the *Picornaviridae.* Hepatitis E virus (HEV), however, has some structural features resembling the caliciviruses, but its unique genomic organization means that it cannot be classified easily within any of the existing virus families.

Both viruses spread predominantly through water and are concentrated by molluscan shellfish. HAV is not uncommon, but HEV is rare in developed countries. It does, however, occur in epidemic form in India and in the former Soviet Union. The worst outbreak of HEV involved 30,000 people in New Delhi in 1955. More limited shellfish-associated outbreaks occur sporadically around the Mediterranean. In the United Kingdom, HEV is limited to returning travelers. Clinical features of both viruses are similar, although HEV tends to be more severe and can be fatal in pregnancy. Convalescence may be prolonged (8–10 weeks), and some 15% of cases of HAV may follow a relapsing course over 12 months or more.

The spread of picornaviruses has been affected profoundly by human activities. In former times, when the quality of water could not be guaranteed, infection occurred early in life through exposure to virus-contaminated water. Under these conditions, infections tended to be mild (often subclinical) and were endemic in the society. However, where water purification and other public health measures have been implemented, the possibilities of infection for all of these viruses are reduced, which has had the effect of increasing the mean interval between contacts with the virus. As a result, infection is delayed, and thus occurs predominantly in older individuals.

This infection delay is well demonstrated in countries where sanitation has improved over recent years.[19,28] In Hong Kong in 1979, 30% of those people under 30 years of age had been infected with HAV; by 1989, this number was 9% and is still falling. Presumably, this will progress eventually to resemble the situation in the developed countries where the vast majority of persons older than 30 have *no* antibody to HAV (i.e., they have never been infected).[18] This is significant for the course of the infection because the virus is more severe if it is contracted in adulthood. People older than 30 years of age account for only some 30% of the cases of HAV, but nearly 80% of the deaths occur in this age range.[19] Shellfish will, of course, concentrate any viruses shed in sewage and contaminating

their natural habitat, including picornaviruses, and perhaps more significantly, HAVs and HEVs. Significant outbreaks of illness have been noted in Singapore and have been attributed directly to virus contamination of shellfish;[47] similar outbreaks that are mainly attributable to direct virus contamination of water occur with HEV.[28] Consequently, as water quality improves, it is expected that persons growing older in the absence of HAV infection would develop a taste for shellfish and shellfish products (including fermented foods) and become exposed to the virus later in their lives. They would have no residual immunity from childhood infections (see astroviruses above), which could pose a risk of severe disease later in life.

HAV is more stable to acid than other gut viruses of the picornavirus family. It retained virtually all activity following 120 minutes at pH 1.0 and was still viable (although reduced in titre) after five to eight hours.[41] Other enteroviruses were inactivated virtually completely within two hours at this pH. HAV is also relatively more heat stable and survives heating at 60 °C for 60 minutes,[10,15,21] being only partially inactivated after 12 hours at this temperature at neutral pH.[36,43] In the absence of divalent cations, 50% of particles will disintegrate within 10 minutes at 61 °C, although in the presence of 1 M MgCl, this is not achieved until 81 °C. The equivalent temperatures for poliovirus are 43 °C and 63 °C, respectively, and polio appears far more sensitive to desiccation and storage in the dry state than HAV. This suggests that poliovirus is an inadequate model for the survival of enteric viruses in general, and especially for HAV.[1,46] HAV does, however, become rapidly inactivated (in minutes) at temperatures higher than 98 °C and can also be destroyed by chlorine and hypochlorite (10–15 ppm residual chlorine after 30 minutes; 3–10 ml/l hypochlorite 20 °C for 5–15 minutes). However, HAV is more chlorine resistant than other picornaviruses.[2,38] In general, HAV is the most stable of the enterically transmitted viruses. It survives desiccation with minimal decrease in titre (0.5 log) and, once dried, shows virtually no reduction in titre during a seven-day period regardless of the surface onto which it has been dried, temperature or relative humidity of storage, or the presence of accompanying fecal material. Titres are reduced by only 1–2 logs during the next 60 days after drying.[1] These data suggest that the survival of HAV is likely in fermented foods, although it has not been rigorously assessed, and exposure to the virus from the consumption of shellfish or fermented foods prepared from them remains a possibility.

ASSESSMENT OF VIRUS RISK

Contamination of Foods

Virus levels will not increase during the fermentation of food; therefore, virus content of these materials becomes an issue only where there is significant contamination of the food before it is fermented. Most food-borne viruses are resistant to both acid conditions and mild heat treatment and could survive these aspects of fermentation processes. NLVs represent the greatest risk of gastroenteritis for adults because they are not likely to be immune. Stability data for these viruses are scarce because of the difficulty in assessing their survival, but it seems unlikely that they would be more resistant to physical treatments than HAV. Thus, in assessing the potential for virus survival in food, it would seem that procedures that would inactivate HAV probably give the greatest protection against virus dissemination in the food. The other viruses that could be present in food are largely infections of childhood. With the exception of fermented cereals and dairy curds in some cultures, children may not eat much fermented food and thus the acquisition of infection from this source (even if the viruses survive) is likely to be rare. Adults consuming food that has been contaminated with these viruses would be protected by residual immunity resulting from childhood infection. HAV poses a serious threat to adults with no preexisting immunity. Because this is the virus that is hardest to remove (and thus most likely to survive), HAV contamination of fermented foods (especially of shellfish-derived foods) could pose an increasing threat in areas

where sanitation is rapidly improving and seropositivity to HAV is falling in adults.

Improved knowledge of the types of food that are regularly contaminated with viruses should permit an assessment of the relative risks associated with particular foods. In the United Kingdom, rigorous investigation often fails to identify the food vehicle of transmission. However, accepting the significance of NLVs as the most commonly identified cause of illness in adults, a recent survey found that 35 (65%) of the NLV outbreaks could be attributed to a particular foodstuff, the most frequent being oysters.[4] In those cases where a particular food was implicated, virus was detected in the food itself in only two cases, both oysters. This percentage reflects the generally low level of virus contamination in other types of food. Other food vehicles implicated were diverse, including sandwiches, pies, fresh fruit, fruit and vegetable salads, gateaux, fish, lobsters, and prawns. Many of the dishes were not served alone, and the common feature could be fresh fruit or salad vegetables that were often served as a garnish if not a main component of the meals. It is not possible to assess how many of these outbreaks were associated with contamination by food handlers immediately before serving, and how many might have resulted from virus contamination of the food before its preparation. Certainly, food handlers were suspected in 19 cases, but was confirmed in only four. Viral illness has been associated with intrinsic contamination of fruit in the past,[6,31] and the worldwide trade in fruit and vegetables may well increase such a risk in the future. It is generally held, in the United Kingdom at least, that there are two major patterns of transmission of food-borne viruses—those due to intrinsic contamination of shellfish with virus, and those due to other foods probably involving contamination by food handlers. It is not generally believed that virus contamination of vegetables and fruit at source is a major contributor to food-borne viral illness in the United Kingdom.

Food-borne spread of HAV is not common. In the United Kingdom, during the 1980s, seven outbreaks were attributed to shellfish and a similar number to food contamination by handlers. More recently, out of 19,000 cases (mostly involving children), only 0.5% were associated with food, with shellfish again being the major culprit. This risk is far more significant elsewhere where substantial outbreaks have been attributed to shellfish consumption. An outbreak associated with clams in Shanghai may have been the largest recorded outbreak of food-borne illness ever.[20] HEV occurs in the United Kingdom only in one or two cases per year, entirely associated with travelers returning from areas in which the disease is endemic. Waterborne spread would seem to be the major vehicle of transmission, although shellfish-associated cases have been reported.

Virus Survival in Fermented Foods

There are few data that are directly concerned with the survival of viruses in fermented foods. Studies conducted in fermented meats have shown that the viruses responsible for foot and mouth disease (i.e., FMDV), African swine fever, and hog cholera do not survive the production process.[29,34] However, these viruses are of veterinary importance and are known to be more labile than human enteric viruses. FMDV is acid labile, is inactivated below pH 6, and is not like gut viruses of the same family such as polio, whereas the viruses causing hog cholera and African swine fever are both enveloped and therefore more susceptible to the adverse conditions encountered during fermentation.[16] When model enteric viruses such as polio, Coxsackie, and echovirus, which are more relevant to human health, were used, they were found to persist in high titres virtually unaffected by the processing that reduced the pH to 5.0–5.4.[12,22,25] Studies with bacteriophage and simian rotavirus in fermented cereals that are more weakly buffered and consequently have a lower pH have also demonstrated the ability of viruses to survive well in fermented products.[32,50] This relative paucity of information does, however, point to the difficulty of controlling risk from food-borne viruses through fermentation, though further work is clearly needed. Based on all of the information available, however, some general infer-

ences can be drawn concerning the risks posed by viruses and their control.

First, the type of food on which the product is based will be an important factor. Shellfish are an obvious high-risk material because they often live in sewage-contaminated estuarine waters and concentrate available viruses in their tissues. The initial preparation of shellfish involves shucking the shellfish flesh, and even if this is then washed, it is unlikely to remove virus contamination from the shellfish gut. Fermented foods prepared from organisms that consume shellfish (such as octopus or scavenging crabs) may be contaminated indirectly from this source. However, viruses are not believed to be retained with the same efficiency in these creatures, so the initial risk is presumably more short lived.

Vegetable produce may be contaminated either on the surface (e.g., by handling, washing, or spraying with contaminated water)[8] or more deeply within the tissues (e.g., resulting from the uptake of viruses contaminating irrigation waters used in cultivation). This is more likely in the case of produce with a high water content, such as celery, pumpkins, cucumbers, and other soft fruits. Surface contamination can be effectively removed by peeling provided the peelings are not simply allowed to elute any virus into the washing water and thus redistribute the material across freshly peeled surfaces. Similarly, washing itself can be very effective, and efficiency can be increased if small quantities of (preferably ionic) detergent can be used.

Where produce is contaminated with fecal material, viruses may be dried on to the surface as aggregates with other organic material. This tends to promote survival on nonporous surfaces such as a waxy plant cuticle. In these cases, the use of detergent could not only remove the aggregate, but also disperse it, thus increasing the number of potential infectious units present. Under these conditions, thorough rinsing becomes essential. Potable water washing alone can reduce surface contamination by bacteria by 10–100-fold, and the use of hypochlorite is even more effective. Common commercial washing procedures in the developed world use 100 ppm hypochlorite (yielding 30–40 ppm free chlorine) at pH 6.8–7.1 and 4 °C and a contact time of two minutes. Other materials such as soft fruits that could be damaged by this process can be sprayed or immersed for only 10 seconds in 15–20 ppm free chlorine.[7] Tests have found that in general, hypochlorite under these conditions is very effective against viruses in suspension;[24] however, its efficacy against viruses that are adsorbed to a surface or in the presence of other organic materials cannot be assumed as great. Studies suggest that hypochlorite may not be as effective as assumed when it is used to control HAV that was artificially introduced on to strawberries. Hypochlorite treatment was approximately 10-fold less effective against this virus on the fruit than the same virus in suspension, and full control could not be achieved below levels that would render the fruit inedible.[45] Furthermore, this method of cleansing must be limited to an effect on surface contamination only; it could clearly not affect any deeper contamination within the plant tissues.

Fermentation frequently involves salting, particularly fermented shellfish products produced in southeast Asia, where salt levels of up to 30% by weight can be used, producing a saturated brine.[39] Salt (sodium chloride) itself is generally not injurious to virus particles lacking an envelope; other ions are more problematic, with cesium having an adverse and sometimes irreversible effect on virus polymerase function. High salt (especially under acid conditions) can lead to the precipitation of proteins; this is likely to occur and will be aided by a generally high protein concentration in the environment. Precipitation on this scale would certainly cause the precipitation of virus particles and a considerable reduction in their infectivity. Precipitated viruses can redissolve should the salt concentration be reduced at a later stage, but residual clumping and loss of infectivity would mean that the original titre will not be recovered. Other salts have a stabilizing effect on virus particles, particularly divalent cations; magnesium stabilizes polioviruses and calcium stabilizes rotaviruses.

pH reduction in fermentation is typical, and pH could fall to as low as 3.8. However, all en-

teric viruses are able to infect through the gut, and thus all must be designed for passage through the acid conditions of the stomach. Indeed, stability to acid (pH 4.0) remains one of the routine tests for enteroviruses, differentiating them from the morphologically identical rhinoviruses that infect via the nasal mucosa and are not required to possess acid stability. Acid resistance is thus a common feature of all of the viruses in these groupings, and acid production *per se* is not likely to reduce virus titre significantly unless accompanied by heat. This is supported by the limited number of studies done on virus survival in acid-fermented foods. Nout *et al.*[32] used the bacteriophage (MS-2) as a model for human viruses and found that it survived much better than bacteria in a fermented porridge at pH 3.8; its numbers declining by approximately 0.1 log cycle per hour. Even better survival was found in work using the simian rotavirus SA 11, a good model for the behavior of unculturable human rotavirus. In 24 hours at 30 °C, the virus titre decreased by 0.25 log cycles at pH 4.0 and by 1 log cycle at pH 3.3.[50] Significantly, this work found the effect to be purely pH-related, with no difference when different acidulants were used. The enhanced antimicrobial effect of weak organic acids seen against bacteria clearly does not operate with nonenveloped, nonmetabolically active virus particles.

During fermentation, autolysis of the tissues will lead to protease release. Gut viruses are required to possess some degree of protease resistance because infection through the gut must expose them to these enzymes. In fact, some seem to have evolved to require the addition of proteases such as trypsin during their culture *in vitro*. Trypsin, however, is a specific enzyme recognizing certain sites in the protein, and all viruses are susceptible to prolonged protease treatment. This is especially true of broad-spectrum proteolytic enzymes such as protease K and pronase, and plant enzymes such as bromelain and papain. The enzymes released during autolysis are also broadly reactive against proteins. Regardless of the context of the peptide bond, they too are able to destroy virus particles eventually. Detergents and saponifiers will also assist this action if present. The addition of protease-containing fruit or fruit juice (especially pineapple) would also aid this effect in fermented shellfish products such as *Plaa-mam* and *Khem-mak-nat*.[39] Virus particles would, however, be protected from this process if they are present as aggregates or inclusions (perhaps within shellfish gut tissue) or surrounded by a high local concentration of protein.

Finally, there is heat treatment. The ability of a heat treatment to eliminate risk from a pathogenic organism will depend not only on the intrinsic heat sensitivity of the organism, but also on the initial numbers of the organism present, the heating menstruum, the temperature, and the time of exposure. Although there is a wealth of information on how these factors can affect bacterial survival, the thermal inactivation kinetics of viruses have not been subject to the same kind of scrutiny. A temperature of 65 °C is ineffective against most of the enteric viruses; certainly astroviruses, HAV, and rotaviruses can survive such treatment even if adenoviruses and polioviruses may be substantially reduced in titre. A recent study[9] has shown that less than 0.5 minutes at 85 °C produced a reduction of more than 5 log cycles in the titre of HAV in three different dairy products, whereas heating at conventional pasteurization temperatures of 71–73 °C required 13–18 minutes to achieve the same effect. Some protective effect from fat was seen in cream containing 18% fat, but no significant differences were discernible between skimmed and whole (3.5% fat) milk.

The failure to see outbreaks of viral illness associated with correctly pasteurized milk indicates the very low association of human viruses with this product. Thus, by extension, fermented milk products are likely to be relatively safe with regard to viral infections, particularly when operations such as milking and fermentation are mechanized, thus reducing the risk of contamination from food handlers. FCV could not be cultured from cockles immersed in boiling water for one minute or longer, during which time the average internal temperature reached 78 °C. Immersion for 30 seconds, which achieved an aver-

age internal temperature of approximately 63 °C, produced a 2-log reduction in titre.[44] HAV (and polio virus) were completely eliminated from cockles that achieved an average internal temperature of 85–90 °C for one minute. These data were used as the basis for setting heat-treatment regulations for cockles in the United Kingdom (90 °C for 90 seconds).[30] In some products, though, the temperature treatment necessary to eliminate risk may be incompatible with product quality, although there is some evidence that in acid (fermented) foods, the heat resistance of viruses is much reduced.[50]

Viruses clearly pose some special problems. The available data suggest that a fermentation process alone will not ensure safety from viral infection, although the highest risk is associated only with a very limited number of substrates. Further studies are clearly indicated to explore the integrated effect of all aspects of processing on virus survival in order to establish a realistic estimate of risk.

REFERENCES

1. Abad, F. X., Pinto, R. M. & Bosch, A. (1994). Survival of enteric viruses on environmental fomites. *Appl Environ Microbiol* 60, 3704–3710.
2. Abad, F. X., Pinto, R. M. & Bosch, A. (1997). Disinfection of human enteric viruses on fomites. *FEMS Microbiol Lett* 156, 107–111.
3. Abad, F. X., Pinto, R. M., Villena, R., Gajardo, R. & Bosch, A. (1997). Astrovirus survival in drinking water. *Appl Environ Microbiol* 63, 3119–3122.
4. Advisory Committee on Microbiological Safety of Food. (1998). *Report on Foodborne Viral Infections*. London: The Stationery Office.
5. Albert, M. J. & Bishop, R. F. (1984). Cultivation of human rotaviruses in cell culture. *J Med Virol* 13, 377–383.
6. Anon (1997). Hepatitis A associated with consumption of frozen strawberries. *WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe* 54, 5.
7. Beuchat, L. R. (1992). Surface disinfection of raw produce. *Dairy, Food Environ San* 12, 6–9.
8. Beuchat, L. R. (1996). Pathogenic microorganisms associated with fresh produce. *J Food Prot.* 59, 204–216.
9. Bidawid, S., Farber, J. M., Sattar, S. A. & Hayward, S. (2000). Heat inactivation of hepatitis A in dairy foods. *J Food Prot* 63, 522–528.
10. Bradley, D. W., Fields, H. A., McCaustland, K. A., Cook, E. H., Gravelle, C. R. & Maynard, J. F. (1978). Biochemical and biophysical characterisation of light and heavy density hepatitis A virus particles: evidence that HAV is an RNA virus. *J Med Virol* 2, 175–187.
11. Carter, M. J. & Cubitt, W. D. (1995). Norwalk and related viruses. *Curr Opinion Infect Dis* 8, 403–409.
12. Dethmers, A. E., Rock, H., Fazio, T. & Johnston, R. W. (1975). Effect of added sodium nitrate on sensory quality and nitrosamine formation in thuringer sausage. *J Food Sci* 40, 491–495.
13. Dolin, R., Blacklow, N. R., DuPont, H., Bushco, R. F., Wyatt, R. G., Kasel, J. A., Hornick, R. & Chanock, R. M. (1972). Biological properties of the Norwalk agent of acute non-bacterial gastroenteritis. *Proc Soc Exp Biol Med* 140, 578–583.
14. Estes, M. K., Graham, D. Y., Smith, E. M. & Gerba, C. P. (1979). Rotavirus stability and inactivation. *J Gen Virol* 43, 403–409.
15. Feinstone, S. M., Moritsugu, Y., Shih, J. W., Gerin, J. L. & Purcell, R. H. (1978). Characterisation of hepatitis A virus. In *Viral Hepatitis*, pp. 41–48. Edited by G. N. Vyas, S. N. Cohen & R. Schmid. Philadelphia, PA: Franklin Institute Press.
16. Fields, B. N. & Knipe, D. M., eds. (1991). *Fundamental Virology*, 2nd edn. New York: Raven Press.
17. Foy, H. M. (1997). Adenoviruses. In *Viral Infections of Humans Epidemiology and Control,* 4th edn., pp. 119–138. Edited by A. S. Evans & R. A. Kaslow. New York: Plenum Medical Book.
18. Frosner, G. G., Papaevagelou, G., Butler, R., Iwarson, S., Lindholm, A., Courouce-Pauty A., Haas, H. and Deinhardt, F. (1979). Antibody against hepatitis A virus in seven European countries: a comparison of prevalence data in different age groups. *Am J Epidemiol* 110, 63–69.
19. Hadler, S. (1992, June). Hepatitis A: changing epidemiology and the need for a vaccine. *Virus and Life* 14–16.
20. Halliday, M. L., Kang, L. Y., Zhou, T. K., Hu, M. D., Pan, Q. C., Fu, T. Y., Huang, Y. S. & Hu, S. L. (1991). An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis* 164, 852–859.
21. Havens, W. P. Jr. (1945). Properties of the etiologic agent of infectious hepatitis. *Proc Soc Exp Biol Med* 58, 203–204.
22. Hermann, J. E. & Cliver, D. O. (1973). Enterovirus persistence in sausage and ground beef. *J Milk Food Technol* 36, 426–428.

23. Hermann, J. E., Hudson, R. W., Blacklow, H. R. & Cubitt, W. D. (1987). Marin county agent: an astrovirus. *Lancet* ii, 743.
24. International Committee for Microbial Safety of Food. (1996). *Micro-Organisms in Foods.* Vol. 5, *Microbiological Specifications of Food Pathogens*. London: Chapman & Hall.
25. Kantor, M. A. & Potter, N. N. (1975). Persistence of echovirus and poliovirus in fermented sausage. *J Food Sci* 40, 491–495.
26. Keswick, B. H., Satterwhite, N. R., Cukor, G. C., DuPont, H. L., Secor, S. L., Bitsure, J. A., Gary, G. W. & Hoff, J. C. (1985). Inactivation of Norwalk virus in drinking water by chlorine. *Appl Environ Microbiol* 50, 261–264.
27. LeBaron, C. W., Furutan, N. P., Lew, J. F., Allen, J. R., Gouvea, V., Moe, C. & Monroe, S. S. (1990). Viral agents of gastroenteritis public health importance and outbreak management. *MMWR* 39, 1–24.
28. Margolis, H. S., Alter, M. J. & Hadler, S. (1997). Viral hepatitis. In *Viral Infections of Humans: Epidemiology and Control,* 4th edn., pp. 363–418. Edited by A. S. Evans & R. A. Kaslow. New York: Plenum Medical Book.
29. McKercher, P. D., Hess, W. R. & Hamdy, F. (1978). Residual viruses in pork products. *Appl Environ Microbiol* 35, 142–145.
30. Millard, J., Appleton, H. & Parry, J. V. (1987). Studies on the ehat inactivation of hepatitis A virus with special reference to shellfish. *Epidemiol Infect* 98, 397–414.
31. Niu, M. T., Polish, L. B., Robertson, B. H., Khanna, B. K., Woodruff, B. A., Shapiro, C. N., Miller, M. A., Smith, J. D., Gedrose, J. K. and Alter, M. J. (1992). Multistate outbreak of hepatitis A associated with frozen strawberries. *J Infect Dis* 166, 518–524.
32. Nout, M. J. R., Rombouts, F. M. & Havelaar, A. (1989). Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic organisms. *Int J Food Microbiol* 8, 351–361.
33. Oishi, I., Yamazaki, K., Kimoto, T., Minekawa, A., Utagawa, E., Yamazaki, T., Inouye, S., Grohman, G. S., Monroe, S. S., Stine, S. E., Carcamo, C., Ando, T. & Glass, R. I. (1994). A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka Japan. *J Infect Dis* 170, 439–443.
34. Panina, G. F., Civardi, A., Massirio, I., Scatozza, F., Baldini, P. & Palmia F. (1989). Survival of foot and mouth disease virus in sausage meat products (Italian salami). *Int J Food Microbiol* 8, 141–148.
35. Parrino, T. A., Schreiber, D. S., Trier, J. S., Kapikian, A. Z. & Blacklow, N. R. (1977). Clinical immunity in acute gastroenteritits caused by the Norwalk agent. *N Engl J Med* 297, 86–89.
36. Parry, J. V. & Mortimer, P. P. (1984). The heat sensitivity of hepatitis A virus determined by a simple tissue culture method. *J Med Virol* 14, 277–283.
37. Parwani, A. V., Saif, L. J. & Kang, S. Y. (1990). Biochemical characterisation of porcine enteric calicivirus: analysis of structural and non-structural viral proteins. *Arch Virol* 112, 41–53.
38. Peterson, D. A., Hurley, T. R. & Wolfe, L. G. (1983). Effect of chlorine treatment on infectivity of hepatitis A virus. *Appl Environ Microbiol* 45, 223–227.
39. Phithakpol, B., Varanyanond, W., Reungmaneepaiton, S. & Wood, H. (1995). *The Traditional Fermented Foods of Thailand.* Bangkok: Institute of Food Research and Product Development.
40. Russel, W. C., Valentine, R. A. & Pereira, H. G. (1967). The effect of heat on the anatomy of adenovirus. *J Gen Virol* 1, 509–522.
41. Scholz, E., Heinricy, U. & Flehmig, B. (1989). Acid stability of hepatitis A virus. *J Gen Virol* 70, 2481–2485.
42. Shirley, J. A. & Beards, G. M. (1981). The influence of divalent cations on the stability of human rotavirus. *Arch Virol* 67, 1–9.
43. Siegl, G., Weitz, M. & Kronauer, G. (1984). Stability of hepatitis A virus. *Intervirology* 22, 218–226.
44. Slomka, M. J. & Appleton, H. (1998). Feline calicivirus as a model system for heat inactivation studies of small round structured viruses in shellfish. *Epidemiol Infect* 121, 401–407.
45. Sobsey, M. (1998). *Surface inactivation of hepatitis A virus on strawberries using chlorine.* Paper presented at the 85th annual meeting of the International Association of Milk, Food and Environmental Sanitarians, October 1998, Nashville TN.
46. Sobsey, M. D., Shields, P. A., Hauchman, F. S., Davis, A. L., Rullman, V. A. & Bosch, A. (1988). Survival and persistence of hepatitis A virus in environmental samples. In *Viral Hepatitis and Liver Disease,* pp. 121–124. Edited by A. J. Zuckerman. New York: Alan R Liss.
47. Tan, Y. W., Wang, J. X., Xu, Z. Y., Guo, Y. F., Qian, W. H. and Xu, J. X. (1991). A serologically confirmed, case-control study, of a large outbreak of hepatitis A in China, associated with consumption of clams. *Epidemiology & Infection* 107, 651–657.
48. Wheeler, J. G., Sethi, D., Cowden, J., Wall, P. G., Rodrigues, L. C., Tompkins, D. S., Hudson, M. J. & Roderick, P. J. (1999). Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. *Br Med J* 318, 1046–1050.
49. Willcocks, M. M. , Laidler, F., Carter, M. J. & Madeley, C. R. (1990). Cultivation of human astrovirus in a continuous cell line. *Arch Virol* 113, 73–82.
50. Wood, G. W. & Adams, M. R. (1992). Effect of acidification, bacterial fermentation and temperature on the survival of rotavirus in a model weaning food. *J Food Prot* 55, 52–55.