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Hepatitis viruses and emerging viruses

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Abstract: This chapter describes viruses that are enterically transmitted and cause systemic disease. These are recognized as important food and waterborne pathogens. The chapter first summarizes the general characteristics of the viruses, then describes their typical epidemiological patterns. The chapter then discusses methods to detect and to inactivate the viruses, with an emphasis on strategies that can be implemented for food safety.

Key words: foodborne viruses, hepatitis A virus, hepatitis E virus, inactivation of viruses, emerging viruses.

25.1 Introduction

Viruses that are recognized as causes of foodborne and waterborne illness can be separated into four general groups: the viruses that cause gastroenteritis (Chapter 32), the viruses that cause hepatitis, the enteroviruses and emerging viruses.

The enterically transmitted hepatitis viruses (hepatitis A and hepatitis E) replicate and cause disease in the liver, while the enteroviruses (polio, echo and coxsackie) replicate in the small intestine but migrate to and cause illness in other organs. These two groups of viruses are transmitted by the faecal–oral route, either directly from person-to-person or indirectly when food or water is contaminated with faecal material from infected individuals. There are also some emerging viruses that are not usually transmitted via the faecal–oral route, but recent reports indicate they may infect via the gastrointestinal tract and have the potential to emerge as a food safety concern (influenza A, coronavirus, tickborne encephalitis). See Table 25.1 for a summary of the viruses discussed in this chapter.

Table 25.1 Summary of enteric viruses and their main characteristics as discussed in this chapter

Virus	Family	Target organs	Illness	Severity
Hepatitis A virus	Picornaviridae	Liver	Hepatitis	Variable, severity increases with age
Hepatitis E virus	Hepeviridae	Liver	Hepatitis	Mild, except in pregnant women
Enteroviruses (Poliovirus) (Echovirus) (Coxsackie virus)	Picornaviridae	Central nervous system, heart, lungs, skin	Poliomyelitis, meningitis, encephalitis, myocarditis, pericarditis, pleurodynia, hand foot and mouth disease	Variable, most infections are mild
Avian Influenza virus (High pathogenicity)	Orthomyxoviridae	Intestine, lungs	Gastroenteritis, respiratory disease	Variable, certain strains have high fatality rates in humans
Coronavirus	Coronaviridae	Intestine, lungs	Gastroenteritis, respiratory disease	Variable, one strain has had high fatality rates in humans
Tick-borne encephalitis virus	Flaviviridae	Central nervous system	Encephalitis	Severe, infections are rare

These organisms are described in the following text, with an emphasis on their potential to cause foodborne illness. Methods to detect and inactivate the major viral pathogens are discussed, with indications for control measures that can be used to limit the spread of viral disease through the food supply. Each virus has particular challenges and considerations, but general themes emerge that can be used to mitigate current and future food safety concerns.

25.2 Description of the organisms

25.2.1 Hepatitis A virus

The hepatitis A virus is a member of the Picornaviridae family, in the genus Hepatovirus (Fauquet *et al.*, 2005). Particles are non-enveloped, icosahedral capsids of approximately 30 nm in diameter (Feinstone *et al.*, 1973). They enclose a 7.5 kb single-stranded, polyadenylated RNA genome that codes for the viral structural and non-structural proteins (Cohen *et al.*, 1987).

The hepatitis A virus infects epithelial cells of the small intestine and hepatocytes in primates (Balayan, 1992). Naturally-occurring disease is seen only in the human population and causes viral hepatitis characterized by fever, jaundice, light coloured stools, dark coloured urine, abdominal pain and occasional diarrhea in older children and adults (Nainan *et al.*, 2006). Infection is generally asymptomatic or anicteric in children under six years of age (Hadler *et al.*, 1980). The infection is acute and its resolution provides lifelong immune protection against future infections, as there is only one serotype of hepatitis A (Jacobsen and Koopman, 2004; Cristina and Costa-Mattioli, 2007).

In developing regions of the world where hygienic standards (i.e. clean water, sewage systems, availability of adequate hygiene facilities and proper hygienic practices) may be below acceptable standards, children are commonly exposed to the hepatitis A virus at an early age. Since most infections in children are asymptomatic, the rates of disease are low and outbreaks are uncommon in these areas (Shapiro and Margolis, 1993). In contrast, in the developed countries, with low hepatitis A endemicity, hepatitis A infections and outbreaks are a serious public health concern (AAPCID, 2007).

The hepatitis A virus is spread via the faecal–oral route, typically by person-to-person contact or by ingestion of contaminated food or water (Brundage and Fitzpatrick, 2006). The virus has a particularly high rate of person-to-person transmission, and it is common for foodborne outbreaks of hepatitis A to be amplified when primary cases transfer the infection to members of their households (Fiore, 2004). The long incubation period between infection and symptomatic disease (15–50 d) also contributes to difficulties in accurately identifying foodborne sources of hepatitis A virus infection (Fiore, 2004). However, a large number of outbreaks have been characterized, and contamination can occur at every stage in food production, from cultivation through final preparation by food handlers.

25.2.2 Hepatitis E virus

The hepatitis E virus (HEV) is a member of the genus *Hepevirus* in the *Hepeviridae* family, which shares features with the *Caliciviridae*, *Togaviridae* and *Picornaviridae* families (Fauquet *et al.*, 2005). The hepatitis E particle is a non-enveloped icosahedron of approximately 30 nm in diameter (Arankalle *et al.*, 1988). The 7.5 kb genome is a single-stranded RNA molecule that codes for three open reading frames (Tam *et al.*, 1991).

A single known serotype of this virus infects humans and animal species; the virus has been isolated from a range of hosts including primates, domestic swine, wild deer, wild boar and birds (Bradley *et al.*, 1987; Meng *et al.*, 1997; Haqshenas *et al.*, 2001; Matsuda *et al.*, 2003; Tei *et al.*, 2003). Anti-HEV antibodies have been detected in an even wider range of host species, including food source animals such as cattle, sheep and goats (Meng, 2000; Lu *et al.*, 2006; Shukla *et al.*, 2007).

The hepatitis E virus causes similar clinical symptoms to those associated with hepatitis A infection. Recent analysis of case studies indicates that there are two clinical forms of hepatitis E infection: a classical type associated with large waterborne outbreaks in the developing world (Acharya and Panda, 2006) and an emerging disease associated with endemically-acquired cases in the developed world (Teo, 2007). This latter form has been detected only sporadically and may be transmitted through contaminated food products.

In classical hepatitis E infections, the cases occur predominantly in young adults (Teo, 2006). The disease presentation is generally more severe than for hepatitis A. Childhood infections are generally symptomatic (Hyams *et al.*, 1992; Tsatsralt-Od *et al.*, 2007). Hyperbilirubinaemia is more pronounced, protracted cholestasis is more common, and a larger proportion of hepatitis E patients present with jaundice (Chau *et al.*, 2006). High (10 % or more) rates of fulminant hepatitis and fatality have been reported for classical hepatitis E infections in pregnant women, particularly in the third trimester (Rab *et al.*, 1997; Boccia *et al.*, 2006).

By contrast, emerging hepatitis E infections in the developed world are more frequently associated with advanced age (Dalton *et al.*, 2007; Teo, 2007). There is no evidence that the water supply is contaminated in the developed world, and this has led to suggestions that such cases may be transmitted through food, although definitive evidence is lacking in almost all cases (Dalton *et al.*, 2007). In these cases, underlying liver disease and excessive alcohol intake are risk factors for fulminant hepatitis, and there is no reported association with pregnancy (Peron *et al.*, 2007).

25.2.3 Poliovirus

Polioviruses are classified in the *Enterovirus* genus of the *Picornaviridae* family (Fauquet *et al.*, 2005). They too form 30 nm particles containing a single-stranded RNA genome (Schwerdt and Fogh, 1957; Young, 1973). The genome is 7.5 kb in length and codes for a single, long open reading

frame that is post-translationally processed to yield all of the viral proteins (Kitamura *et al.*, 1981).

Infection with poliovirus occurs following the ingestion of contaminated food or water. The virus replicates in the human intestinal tract resulting in asymptomatic infection or minor malaise in over 90 % of cases (Melnick, 1996). Mild illness characterized by fever, headache, nausea and sore throat can occur in 4–8 % of infections. If the virus spreads to infect the nervous system, a further 1–2 % of cases experience stiffness in the back and neck, as well as mild muscle weakness. The major illness, paralytic poliomyelitis, occurs in approximately 1 % of cases and consists of meningitis plus persisting weakness of one or more muscle groups. The amount of damage to neurons is highly variable (Melnick, 1996). In endemic countries, the virus affects predominantly young children, with most cases of poliomyelitis occurring in those below five years of age (Singh *et al.*, 1996).

Poliovirus was the first virus shown to be transmitted through food, and a number of outbreaks were associated with raw milk prior to the introduction of routine pasteurization (Dingman, 1916; Sullivan and Read, 1968). Polio can be prevented using a live attenuated vaccine administered orally or by an injectable killed vaccine (Melnick, 1996). As a result of the poliovirus eradication campaign led by the World Health Organization (WHO), infection with this virus has been eradicated or significantly reduced in many regions of the world and, as such, it is of questionable importance for routine food safety considerations (Lahariya, 2007). However, due to its historic importance and the early development of an attenuated vaccine strain, there have been many studies on the spread and inactivation of poliovirus in food products.

25.2.4 Avian Influenza virus

The Influenza virus A genus is classified in the family Orthomyxoviridae (Fauquet *et al.*, 2005). This family is characterized by pleomorphic, enveloped virions with a segmented single-stranded RNA genome (Fauquet *et al.*, 2005). The genome is 14 kb of negative-sense RNA in eight segments, and its complement codes for structural and non-structural proteins (Fauquet *et al.*, 2005). They are classified into sub-types on the basis of the two envelope glycoproteins, the hemagglutinin (H type) and the neuraminidase (N type) (Knossow and Skehel, 2006). There are 16 known H types and 9 known N types, which can theoretically be mixed and matched in all possible combinations (Knossow and Skehel, 2006).

All of the known sub-types of Influenza A viruses (82 H/N combinations out of a possible 144) have been found in wild birds (Van Reeth, 2007). Viruses containing combinations of the H1, H2, H3, N1 and N2 types are considered to be established in the human population; i.e. there are circulating strains of these viruses that predominantly infect humans (Hay *et al.*, 2001). In addition, viruses of the H5, H7 and H9 sub-types have been found to cause sporadic human infections (Peiris *et al.*, 1999; Shortridge *et al.*, 2000;

Koopmans *et al.*, 2004). The subtype of recent worldwide concern is the highly pathogenic H5N1 virus (Abdel-Ghafar *et al.*, 2008). This particular avian influenza virus sub-type has been detected in poultry from over 50 countries on three continents (Van Reeth, 2007). It has infected humans in Vietnam, Thailand, Indonesia, Cambodia, China, Turkey, Iraq, Azerbaijan, Egypt and Djibouti (de Jong and Hien, 2006). This virus replicates to abnormally high levels in the upper respiratory tract, causing an intense inflammatory response and an extremely high case fatality rate of over 60 % (de Jong *et al.*, 2006). The H5N1 avian influenza virus currently has a limited ability to spread from person to person. However, there is concern that this virus could acquire the ability to spread effectively in humans, leading to a worldwide pandemic (Rajagopal and Treanor, 2007). The Spanish influenza pandemic, which killed approximately 50 million people worldwide is thought to have arisen from an avian H1N1 strain (Rajagopal and Treanor, 2007).

To date, H5N1 avian influenza infections of humans have nearly all been linked to close contact between the afflicted individuals and infected poultry (de Jong and Hien, 2006; Van Reeth, 2007; Abdel-Ghafar *et al.*, 2008). However, the virus can be isolated from all parts of infected poultry including the blood, bones and meat (Lu *et al.*, 2003b; Swayne, 2006a). As a result, the consumption of raw or undercooked poultry products represents a potential source of infection (Swayne, 2006b).

25.2.5 Coronavirus

The members of the Coronaviridae family are pleiomorphic, enveloped, single-stranded RNA viruses (Fauquet *et al.*, 2005). The positive-sense, 30 kb genome codes directly for viral proteins (Fauquet *et al.*, 2005). These viruses typically cause mild respiratory disease; however, a particularly virulent strain known as the Sudden Acute Respiratory Syndrome Coronavirus (SARS-CoV) emerged in 2003 and caused over 8000 cases, with a nearly 10 % case fatality rate (Wang and Chang, 2004). This virus caused systematic infections as well as respiratory illness, and was identified in the digestive tract, as well as in faeces and sewage (Chan-Yeung and Xu, 2003; Zhang, 2003; Wang *et al.*, 2005). This raises the possibility that the virus may have had the potential to spread through the faecal–oral route and food products. Although these viruses are able to persist for days in some buffered media, they are sensitive to heating, UV light and disinfection (Duan *et al.*, 2003; Rabenau *et al.*, 2005a, b). No cases of foodborne spread were documented during this outbreak, although one cluster of cases in a housing complex was linked to an index patient suffering from diarrhoea (McKinney *et al.*, 2006).

25.2.6 Other enteroviruses

The echoviruses and coxsackieviruses are enteroviruses of the family Picornaviridae and share many features with the polioviruses. They have 30

nm, non-enveloped particles enclosing a positive-sense single-stranded RNA genome (Fauquet *et al.*, 2005). These viruses are common and infections are mostly asymptomatic. They can occasionally spread outside of the gastrointestinal tract to cause aseptic meningitis, myocarditis or encephalitis. Infection of newborns with these viruses can lead to serious or fatal infection (Abzug, 2004). These viruses are transmitted via the faecal–oral route, but are not frequently associated with foodborne illness. A recent report identified milk from an infected mother as the source of coxsackievirus B infection (Chang *et al.*, 2006).

25.2.7 Tickborne Encephalitis virus

The tickborne encephalitis virus is an enveloped, single-stranded RNA virus of the family Flaviviridae (Fauquet *et al.*, 2005). It is endemic to Europe and Russia, causing 10 000–12 000 cases of encephalitis every year, with a case fatality rate of 0.5 % (Gunther and Haglund, 2005). Survivors can develop long-term neurological sequelae at rates of up to 40 % (Dumpis *et al.*, 1999). The majority of cases are transmitted by tick bites, but some have been associated with the consumption of raw milk from infected cattle or goats (Sixl *et al.*, 1989; WHO, 1994; Dumpis *et al.*, 1999; Kerbo *et al.*, 2005).

25.2.8 Emerging viruses of gastroenteritis

Additional emerging viruses of interest cause symptoms of gastroenteritis. These include the parvoviruses, toroviruses and picobirnaviruses. See Chapter 32 for a detailed description of the causative agents of viral gastroenteritis.

25.3 Risk factors

25.3.1 Hepatitis A virus

There is an inverse correlation between hepatitis A disease rates and viral endemicity (Shapiro and Margolis, 1993). The Centres for Disease Control and Prevention in the USA has classified regions according to high (Central and South America, Africa, South Asia, Greenland), intermediate (Russia and Eastern Europe) and low (North America, Western Europe, Scandinavia, Australia) seroprevalence rates for hepatitis A (CDC, 2007). This is important because many of the cases and outbreaks of hepatitis A in developed countries can be traced to travel in endemic countries (Steffen, 2005). There may also be a higher risk for food produced in the highly endemic countries to be contaminated with the hepatitis A virus (Fiore, 2004).

Disease rates in low endemicity countries are difficult to generalize. They are characterized by cyclical peaks and troughs, and they are changing in response to recent vaccination campaigns (Pham *et al.*, 2005; Wasley *et al.*,

2005). Undiagnosed children may be an important source of the hepatitis A virus in communities, as a high percentage of hepatitis A infections have no known source (Staes *et al.*, 2000; Nainan *et al.*, 2005). There has been a safe and effective vaccine available against hepatitis A since the mid-1990s; however, this virus continues to cause outbreaks, with contaminated food being one important source of infection (Fiore *et al.*, 2006; Craig *et al.*, 2007).

Foodborne outbreaks of hepatitis A have been associated with many different food types and different settings (see Table 25.2 for a summary of some outbreaks published over the past 10 years). Viruses are inert particles when present outside their host. The spread of hepatitis A infection through contaminated food, water or fomites is therefore dependent on the persistence of the virus after the introduction of contaminated human waste.

Documented outbreaks of hepatitis A due to shellfish have been caused by faecal contamination of shellfish growing waters (Conaty *et al.*, 2000; Bosch *et al.*, 2001; Bialek *et al.*, 2007; Pontrelli *et al.*, 2007; Shieh *et al.*, 2007). The hepatitis A virus survives for up to one month in dried faecal matter (McCaustland *et al.*, 1982). In mixtures of human and animal waste designed for waste treatment prior to disposal, it takes 7 d to reduce viral titre by 1 log₁₀ at 37 °C (Deng and Cliver, 1995). Once introduced into the water environment, the hepatitis A virus also remains infectious for months, associating with marine sediment (Arnal *et al.*, 1998; Bosch, 1998). The hepatitis A virus has been shown experimentally to accumulate in mussels and oysters, and depuration is not effective at eliminating the virus from the shellfish tissue (Franco *et al.*, 1990; Enriquez *et al.*, 1992; Abad *et al.*, 1997b; De Medici *et al.*, 2001; Kingsley and Richards, 2003). Extensive depuration for more than three weeks was required to completely eliminate detectable hepatitis A virus from oyster tissue (Kingsley and Richards, 2003).

Fresh or frozen produce is another important source of foodborne hepatitis A outbreaks (Hutin *et al.*, 1999; Dentinger *et al.*, 2001; Calder *et al.*, 2003; CDC, 2003b; Amon *et al.*, 2005; Wheeler *et al.*, 2005; Frank *et al.*, 2007). In these reports, the human fecal contamination might have been introduced during produce growth (Amon *et al.*, 2005), harvest (Calder *et al.*, 2003), processing (Frank *et al.*, 2007) or distribution. Consequently, the point of viral contamination can be difficult to identify (Hutin *et al.*, 1999; Dentinger *et al.*, 2001; CDC, 2003b; Howitz *et al.*, 2005; Wheeler *et al.*, 2005; Tekeuchi *et al.*, 2006). Once fresh produce is contaminated, the hepatitis A virus adsorbs to its surface and persists for days (Crocì *et al.*, 2002; Stine *et al.*, 2005). Freezing allows the virus to survive for months to years (Niu *et al.*, 1992). In addition, washing the contaminated produce does not usually substantially reduce the level of contamination (Crocì *et al.*, 2002).

The remaining source of reported hepatitis A outbreaks are infected food handlers (CDC, 2003a; Prato *et al.*, 2006; Schenkel *et al.*, 2006; Hasegawa *et al.*, 2007). Hepatitis A virus has been shown to persist on experimentally-contaminated hands for more than 4 h (Mbithi *et al.*, 1992). The virus was

Table 25.2 Selected foodborne outbreaks of hepatitis A virus

Year	# of cases	Country	Food type	Contamination	Reference
1997	467	Australia	Oysters	Growth water	Conaty <i>et al.</i> , 2000
1997	256	USA	Frozen strawberries	Unidentified	Hutin <i>et al.</i> , 1999
1998	43	USA	Green onions	Prior to restaurant delivery (unknown)	Dentinger <i>et al.</i> , 2001
2001	183	Spain	Clams	Growth water	Bosch <i>et al.</i> , 2001
2001	46	USA	Sandwiches	Food handler	CDC, 2003a
2002	26	Italy	Various	Food handler	Prato <i>et al.</i> , 2006
2002	43	New Zealand	Raw blueberries	Harvest	Calder <i>et al.</i> , 2003
2003	601	USA	Green onions	Prior to restaurant delivery (unknown)	CDC, 2003b; Wheeler <i>et al.</i> , 2005
2003	422	USA	Green onions	Farm	Amon <i>et al.</i> , 2005
2004	64	Germany	Baked goods	Food handler	Schenkel <i>et al.</i> , 2006
2004	1180	India	Water	Sewage contamination	Arankalle <i>et al.</i> , 2006
2004	884	Italy	Raw shellfish	Growth water	Pontrelli <i>et al.</i> , 2007
2004	351	Egypt	Orange juice	Processing plant	Frank <i>et al.</i> , 2007
2005	4	Denmark	Ice cream	Unknown	Howitz <i>et al.</i> , 2005
2005	39	USA	Oysters	Growth water	Bialek <i>et al.</i> , 2007; Shieh <i>et al.</i> , 2007
2006	9	Japan	Sushi bar (undetermined)	Unknown	Tekeuchi <i>et al.</i> , 2006
2006	15	Japan	Restaurant (undetermined)	Food handler	Hasegawa <i>et al.</i> , 2007

readily transferred between inoculated fingers, to foods or stainless steel surfaces and from stainless steel surfaces (Mbithi *et al.*, 1992; Bidawid *et al.*, 2000a). The virus has been shown to attach to many of the surfaces common in food preparation settings, i.e. stainless steel, copper, polythene and polyvinyl chloride (Kukavica-Ibrulj *et al.*, 2004) and to survive for more than 60 d on aluminium, china, latex and paper surfaces (Abad *et al.*, 1994).

The most effective prevention of hepatitis A virus transmission is to prevent faecal contamination of foods and food preparation surfaces (see Section 25.5.1). In addition, there are some physical and chemical procedures known to effectively eliminate the hepatitis A virus from contaminated foods and surfaces (Tables 25.3 and 25.4)

The hepatitis A virus is resistant to acid treatment, both in buffered solutions and in food products (Scholz *et al.*, 1989; Hewitt and Greening, 2004). Heating is an effective mode of viral inactivation, but higher temperatures than those generally used to reduce bacterial counts are required (Parry and Mortimer, 1984). Protection of the virus is also conferred by food matrices, and longer times and/or higher temperatures are required to completely inactivate the virus (Crocchi *et al.*, 1999; Bidawid *et al.*, 2000d; Deboosere *et al.*, 2004). For example, increasing the fat content (Bidawid *et al.*, 2000d) or the sucrose concentration (Deboosere *et al.*, 2004) of food preparations has been shown to increase the heat resistance of the virus. This may be due to a general effect of decreasing the water activity, but this possibility has not been systematically addressed. The result is that many traditional heat-inactivation or cooking protocols, such as pasteurization, steaming or baking, are insufficient to ensure the safety of hepatitis A-contaminated foods (Bidawid *et al.*, 2000d; Crocchi *et al.*, 2005; Hewitt and Greening, 2006).

Heating to 85 °C is not possible for fresh fruits and vegetables, and even for milk and shellfish the procedures required may render foods unpalatable. Surface decontamination may be possible using targeted applications. Alternative physical inactivation methods show some promise for the inactivation of hepatitis A in food products. The virus is sensitive to UV light, but the light must access viral particles to inactivate them, which makes the technique difficult to apply to most food products (Nuanualsuwan *et al.*, 2002). High hydrostatic pressure is a newer technique that has the potential to inactivate the hepatitis A virus in food products while maintaining the organoleptic properties of raw products, particularly shellfish. The technique shows promise for the inactivation of hepatitis A virus in buffer, oysters, strawberries and green onions, although high pressure may reduce the palatability of fresh fruits and vegetables (Kingsley *et al.*, 2002, 2005; Calci *et al.*, 2005).

Chemical methods are frequently used to eliminate virus dried on hands or on surfaces. The hepatitis A virus is more resistant to chemical disinfection than many enteric bacteria and enveloped viruses. Many commercially-available disinfectants are not effective when used on hepatitis A virus-contaminated

Table 25.3 Physical inactivation of enteric viruses discussed in this chapter

Virus	Matrix	Treatment	Time	Log reduction	Conclusion	Reference
Hepatitis A virus	Buffer	pH 1.0 (RT ^a)	5 h	5	Acid stable	Scholz <i>et al.</i> , 1989
	Mussels	pH 3.75 (4 °C)	4 wk	None	Acid stable	Hewitt and Greening, 2004
	Buffer (pH 7.4)	75 °C	30 s	5	Inactivated by high heat	Parry and Mortimer, 1984
	Milk	75 °C	7 min	5	Protected by matrix	Bidawid <i>et al.</i> , 2000d
	Mussels	80 °C	15 min	5	Protected by matrix	Croci <i>et al.</i> , 1999
	Strawberry mash	85 °C	10 min	5	Protected by matrix	Deboosere <i>et al.</i> , 2004
	Buffer (pH 7.4)	36.5 mW/cm ² UV light (RT)	1 s	1	UV sensitive	Nuanualsuwan <i>et al.</i> , 2002
	Strawberries	3 kGy gamma irradiation (RT)	N/A	1	Higher dose than regulators allow	Bidawid <i>et al.</i> , 2000b
	Buffer (pH 7.4)	450 MPa (RT)	5 min	7	Pressure-sensitive	Kingsley <i>et al.</i> , 2002
	Oysters	400 MPa (9 °C)	1 min	3	Potential for use in matrix	Calci <i>et al.</i> , 2005
	Strawberry puree	375 MPa (RT)	5 min	4	Potential for use in matrix	Kingsley <i>et al.</i> , 2005
Green onions	375 MPa (RT)	5 min	5	Potential for use in matrix	Kingsley <i>et al.</i> , 2005	
Hepatitis E virus	Buffer (pH 7.4)	56 °C	60 min	2	Moderately heat-resistant	Emerson <i>et al.</i> , 2005
	Liver	56 °C	60 min	Incomplete ^b	Moderately heat-resistant	Feagins <i>et al.</i> , 2008
	Liver	71 °C	5 min	Complete ^b	Moderately heat-resistant	Zafrullah <i>et al.</i> , 2004
Poliovirus	Buffer	pH 1.0 (RT)	1 min	4	Unstable	Scholz <i>et al.</i> , 1989
	Buffer	pH 3.0 (RT)	30 min	0	Moderately acid stable	Eubanks and Farrah, 1981
	Buffer (pH 7.4)	50 °C	2 min	4	Heat-sensitive	McGregor and Mayor, 1971
	Septage	55 °C	15 min	5	Heat-sensitive	Stramer and Cliver, 1984
	Milk	72 °C	15 s	<4	Resistant to some pasteurization	Strazynski <i>et al.</i> , 2002
	Yoghurt	72 °C	15 s	<4	Resistant to some pasteurization	Strazynski <i>et al.</i> , 2002
	Buffer (pH 7.4)	24 mW/cm ² UV light (RT)	1 s	0.5–1	UV sensitive	Ma <i>et al.</i> , 1994; Nuanualsuwan <i>et al.</i> , 2002

Table 25.3 (Cont'd)

Virus	Matrix	Treatment	Time	Log reduction	Conclusion	Reference
Avian Influenza virus	Buffer	pH 2.0 (RT)	5 min	5	Acid labile	Lu <i>et al.</i> , 2003a
	Buffer	pH 5.0 (RT)	15 min	0	Stable	Lu <i>et al.</i> , 2003a
	Buffer (pH 7.4)	63 °C	2 min	5	Heat-sensitive	Isbarn <i>et al.</i> , 2007
	Whole egg	63 °C	2 min	5	Heat-sensitive	Swayne and Beck, 2004
	Dried egg white	63 °C	1 d	5	Protected by matrix	Swayne and Beck, 2004
	Chicken meat	70 °C	2 s	5	Heat-sensitive	Thomas and Swayne, 2007
	Buffer (pH 7.4)	500 MPa (15 °C)	15 s	5	Pressure-sensitive	Isbarn <i>et al.</i> , 2007
SARS coronavirus	Buffer (pH 7.4)	56 °C	30 min	6	Heat-sensitive	Duan <i>et al.</i> , 2003; Rabenau <i>et al.</i> , 2005a
	Buffer (pH 7.4) + 20 % fetal calf serum	56 °C	30 min	2	Protein protects virus	Rabenau <i>et al.</i> , 2005a
	Buffer (pH 7.4) + 20 % fetal calf serum	60 °C	30 min	6	Heat-sensitive	Rabenau <i>et al.</i> , 2005a
	Buffer (pH 7.4)	90 µW/cm ² UV light (RT)	60 min	6	UV-sensitive	Duan <i>et al.</i> , 2003

^a Room temperature.^b Initial titre of virus stock not available.

Table 25.4 Chemical disinfection/inactivation of enteric viruses discussed in this chapter

Virus	Active compound	Contact time	Log reduction	Conclusion	Reference
Hepatitis A virus	Quaternary ammonium	5 min	< 2	Not effective	Mbithi <i>et al.</i> , 1990 (0.1–2.7 %); Jean <i>et al.</i> , 2003 (10 %)
	Glutaraldehyde	1 min	> 4	Effective	Mbithi <i>et al.</i> , 1990 (2 %)
	Sodium hydroxide	1 min	1	Not effective	Terpstra <i>et al.</i> , 2007 (0.1 N)
	Ethanol	1–5 min	< 1	Not effective	Abad <i>et al.</i> , 1997a (70 %); Mbithi <i>et al.</i> , 1990 (70 %); Bidawid <i>et al.</i> , 2000a (75 %), van Engelenburg <i>et al.</i> , 2002 (80 %)
	Iodide	1–5 min	< 1	Not effective	Mbithi <i>et al.</i> , 1990 (0.07 %, 75 ppm); Jean <i>et al.</i> , 2003 (2 %)
	Phenol	1 min	1–2	Not effective	Mbithi <i>et al.</i> , 1990 (0.1 %), Abad <i>et al.</i> , 1997a (1.41 %)
	Sodium hypochlorite	1 min	< 1–5	5000 ppm free chlorine and full 1 minute contact time are essential	Grabow <i>et al.</i> , 1983 (0.4 mg/l); Abad <i>et al.</i> , 1997a (0.125 %); Jean <i>et al.</i> , 2003 (12 %); Terpstra <i>et al.</i> , 2007 (0.1 %, 1000 ppm); Mbithi <i>et al.</i> , 1990 (5000 ppm)
	Sodium chlorite	1 min	< 1–3	Time and concentration dependent, not as effective as sodium hypochlorite	Mbithi <i>et al.</i> , 1990 (0.23 %); Abad <i>et al.</i> , 1997a (30 %)
	Hypochlorous acid	1 min	< 1	Not effective	Bigliardi and Sansebastiano, 2006 (1.6 mg/l)
Hepatitis E virus	Sodium hypochlorite	30 min	Incomplete	0.6 ppm free chlorine residual in drinking water is not effective	Guthmann <i>et al.</i> , 2006 (0.6 mg/l)

Table 25.4 (Cont'd)

Virus	Active compound	Contact time	Log reduction	Conclusion	Reference
Poliovirus	Quaternary ammonium	30 s		Effective	Weber <i>et al.</i> , 1999 (0.06 %)
	Glutaraldehyde	3 h	5	Effective	Stramer and Cliver, 1984 (1 mg/ml)
	Ethanol	1–2 min	1–4	Moderately effective	Kramer <i>et al.</i> , 2006 (70 %); Mbithi <i>et al.</i> , 1993 (62 %); Abad <i>et al.</i> , 1997a (70 %)
	Phenol	1 min	<1	Not effective	Weber <i>et al.</i> , 1999 (0.06 %); Abad <i>et al.</i> , 1997a (1.41 %)
	Sodium hypochlorite	1 min	4	Effective	Weber <i>et al.</i> , 1999 (5000 ppm); Ma <i>et al.</i> , 1994 (0.5 mg/l); Lukasik <i>et al.</i> , 2003 (300 ppm); Abad <i>et al.</i> , 1997a (0.12 %)
	Sodium chlorite Hypochlorous acid	1 min 1 min	< 1 < 1	Not effective Not effective	Abad <i>et al.</i> , 1997a (30 %) Bigliardi and Sansebastiano, 2006 (1.6 mg/l)
Avian Influenza virus	Quaternary ammonium	1 min	5	Effective	Suarez <i>et al.</i> , 2003 (0.06 %)
	Formaldehyde	1 min	5	Effective	King, 1991 (0.04 %)
	Ethanol	15 min	5	Effective	Lu <i>et al.</i> , 2003a (70 %)
	Phenol	1 min	5	Effective	Suarez <i>et al.</i> , 2003 (0.06 %)
	Sodium hypochlorite	1 min	5	Effective	Suarez <i>et al.</i> , 2003 (0.4 %)
SARS coronavirus	Ethanol	15 s	4	Effective	Rabenau <i>et al.</i> , 2005a (2 %); Rabenau <i>et al.</i> , 2005b (2 %)
	Benzalkonium chloride	30 min	4	Effective	Rabenau <i>et al.</i> , 2005b (0.5 %);
	Glutaraldehyde	15 min	4	Effective	Rabenau <i>et al.</i> , 2005a (0.5 %); Rabenau <i>et al.</i> , 2005b (0.5 %)

surfaces (see Table 25.4) (Mbithi *et al.*, 1990; Abad *et al.*, 1997a; Bidawid *et al.*, 2000a; van Engelenburg *et al.*, 2002; Jean *et al.*, 2003; Bigliardi and Sansebastiano, 2006; Terpstra *et al.*, 2007). Notably, the active ethanol component of many commercial hand disinfectants has a very limited ability to reduce infectious hepatitis A virus titre from contaminated hands or surfaces (Mbithi *et al.*, 1990; Bidawid *et al.*, 2000a). The most effective disinfectants are 2 % glutaraldehyde and 10 % sodium hypochlorite (5000 ppm free chlorine) (Grabow *et al.*, 1983; Mbithi *et al.*, 1990; Abad *et al.*, 1997a; Jean *et al.*, 2003). Care must always be taken to follow appropriate time/concentration combinations for effective disinfection.

25.3.2 Hepatitis E virus

The hepatitis E virus is considered to be an endemic human pathogen in Central America, North Africa and South Asia (Panda *et al.*, 2007). It causes epidemic outbreaks of acute, enterically transmitted hepatitis in these countries, usually associated with faecally-contaminated water (Rab *et al.*, 1997; Guthmann *et al.*, 2006; Panda *et al.*, 2007). Such outbreaks have been particularly associated with the Indian subcontinent, although this may reflect a reporting bias rather than the true level of incidence (Panda *et al.*, 2007).

During these classical outbreaks in endemic countries, person-to-person transmission does not contribute greatly to the number of cases (Hla *et al.*, 1985; Somani *et al.*, 2003). The viral genome has been detected in raw and treated sewage, and outbreaks have been associated with chlorinated water supplies, indicating that basic treatment designed to eliminate faecal coliforms may not be sufficient to inactivate the hepatitis E virus (Jothikumar *et al.*, 1993; Guthmann *et al.*, 2006). Sporadic cases of hepatitis E infection also occur in endemic countries and are likely contribute to the spread of the virus in the population (Nanda *et al.*, 1994).

In Western Europe, North America, Japan and Australia, the hepatitis E virus is an emerging concern (Teo, 2006). Hepatitis E infections in these countries have historically been associated with travel to endemic regions (Zaaijer *et al.*, 1993; Skidmore and Sherratt, 1996). Recently, however, there have been reports of locally-acquired cases in developed countries, but the source of these infections remains uncertain (Mansuy *et al.*, 2004; Ijaz *et al.*, 2005; Waar *et al.*, 2005; Reuter *et al.*, 2006; Dalton *et al.*, 2007; Perez-Gracia *et al.*, 2007; Peron *et al.*, 2007). In two cases, the disease has been linked to the consumption of raw or undercooked meat from animals naturally infected with hepatitis E (Matsuda *et al.*, 2003; Tei *et al.*, 2003). This has led to the hypothesis that the hepatitis E virus is an emerging foodborne zoonosis in developed nations (Teo, 2006).

The hepatitis E virus is known to infect a wide range of animal species (Goens and Perdue, 2004; Vasickova *et al.*, 2007). Most of the studies and evidence for zoonotic transmission to humans have focused on strains isolated

from swine (Goens and Perdue, 2004). Studies have shown that these strains are very closely related to the human viruses and that they productively infect primates (Meng *et al.*, 1997, 1998). Phylogenetic analysis indicates that swine viruses are more closely related to their human counterparts within a country than to other swine isolates around the world (Meng *et al.*, 1997; Hsieh *et al.*, 1999; van der Poel *et al.*, 2001; Pei and Yoo, 2002; Nishizawa *et al.*, 2003; Banks *et al.*, 2004). Zoonotic transmission of this virus to the human population has been postulated to occur through the consumption of raw or lightly cooked meat from a naturally infected animal (Teo, 2006; Vasickova *et al.*, 2007). The evidence for this is strong in a few cases in Japan, but weak elsewhere (Matsuda *et al.*, 2003; Tei *et al.*, 2003). However, infectious hepatitis E virus and/or viral RNA has been isolated from commercial pig livers in the USA, the Netherlands and Japan, indicating that this is a possible transmission route (Yazaki *et al.*, 2003; Bouwknegt *et al.*, 2007; Feagins *et al.*, 2007).

Testing the physical and chemical stability of the hepatitis E virus is challenging due to the lack of a cell culture system to propagate the virus. As such, the pH stability of this virus has yet to be formally demonstrated. The capsid protein acquires an increased heat stability at low pH, and the virus is known to infect via the gastrointestinal tract, implying some resistance to acidic conditions (Zafrullah *et al.*, 2004). The virus resists inactivation by heating at 56 °C, but heating to 71 °C completely inactivated the virus in naturally-contaminated pig livers (Emerson *et al.*, 2005; Feagins *et al.*, 2008). Other physical and chemical methods of inactivation have not been tested, although an outbreak linked to chlorinated drinking water indicates that free chlorine residuals known to reduce fecal coliforms are not sufficient to inactivate the hepatitis E virus (Guthmann *et al.*, 2006).

25.3.3 Poliovirus

The poliovirus is no longer a prevalent enteric pathogen around the world. The widespread use of the oral polio vaccine, which confers intestinal immunity, has led to the eradication of the virus from three of the six regions of the world as defined by the WHO (Sabin, 1991; Lahariya, 2007). These three regions are: the Americas, representing 35 countries, polio-free since 1994; the Western Pacific, representing 37 countries and territories, polio-free since 2000; and the European, representing 51 countries, polio-free since 2002 (CDC, 1994, 2001, 2002). The disease is still endemic in Afghanistan, India, Nigeria and Pakistan (Lahariya, 2007). In recent years, there have also been other countries within the WHO regions of Africa, South-East Asia and the Eastern Mediterranean that have experienced outbreaks of poliomyelitis caused by both wild and vaccine-derived strains (Melnick, 1996; Chumakov *et al.*, 2007). Due to the prevalence of asymptomatic disease, the absence of poliomyelitis does not always correlate with the absence of the poliovirus, and intensive vaccination campaigns are still warranted (Chumakov *et al.*,

2007). Individuals in polio-free countries still routinely follow a course of vaccination with the killed vaccine (Wood *et al.*, 2000, 2006).

Similarly to the hepatitis A virus, the poliovirus can be introduced at any stage in the farm-to-fork continuum. The virus has a low infectious dose (Plotkin *et al.*, 1959) and is frequently isolated from sewage during outbreaks and in endemic countries (Arya and Agarwal, 2007). Once again, shellfish, fresh produce and food handlers are the most significant sources of foodborne virus transmission.

Poliovirus accumulates readily in shellfish and concentrates in the digestive tract (Di Girolamo *et al.*, 1975). Depuration of poliovirus is more effective than for hepatitis A, and most of the virus can be eliminated in free-flow depuration system after 5 d (Di Girolamo *et al.*, 1975; Franco *et al.*, 1990; Enriquez *et al.*, 1992).

The stability and accumulation of poliovirus is of significant concern in agricultural systems. The poliovirus is inactivated more readily than the hepatitis A virus by heating and storage treatments used to prepare manure for spreading on lands (Stramer and Cliver, 1984; Deng and Cliver, 1992). However, once the environment is contaminated, the poliovirus survives for weeks to months in groundwater (Yates *et al.*, 1985; Gordon and Toze, 2003) and in soil (Yeager and O'Brien, 1979; Hurst *et al.*, 1980). This virus has also been shown to persist for weeks to months on vegetables irrigated by spraying or flooding with contaminated waters (Tierney *et al.*, 1977). It has also been demonstrated to survive for weeks to months on fresh and frozen produce, and simple washing does not appear to effectively eliminate poliovirus from food surfaces (Kurdziel *et al.*, 2001; Lukasik *et al.*, 2003).

Poliovirus is moderately acid stable, resistant to incubation at pH 3.0 for 30 min, but sensitive to pH 1.0 (Eubanks and Farrah, 1981; Siegl *et al.*, 1984; Scholz *et al.*, 1989). Heating is extremely effective for the inactivation of poliovirus in buffered solutions, although food matrices may provide some protection and require longer heating times (see Table 25.3) (McGregor and Mayor, 1971; Milo, 1971; Stramer and Cliver, 1984; Strazynski *et al.*, 2002). This may be due to protection provided by protein, fat, lowered a_w or a combination of these parameters. Both UV light and ozone are effective in elimination of the poliovirus, and have been considered for the treatment of wastewater to be used in irrigation (Ma *et al.*, 1994; Nuanualsuwan *et al.*, 2002; Lazarova and Savoys, 2004; Tanner *et al.*, 2004).

Food handlers with inadequate hygiene are frequently implicated in the transmission of viruses via the fecal–oral route. The virus can be transferred from contaminated hands to surfaces, where it resists drying and can persist for days to weeks (Mbithi *et al.*, 1993; Abad *et al.*, 2001). In a food service environment, there are a number of chemicals available to disinfect hands and surfaces potentially contaminated with the poliovirus (Table 25.4). Reagents that implement quaternary ammoniums, glutaraldehyde or sodium hypochlorite as the active ingredient are effective against poliovirus on surfaces (Stramer and Cliver, 1984; Ma *et al.*, 1994; Abad *et al.*, 1997a; Weber *et al.*, 1999;

Lukasik *et al.*, 2003). The poliovirus is removed by soap and water hand-washing for 5 min, but ethanol-based hand disinfectants (60–70 % ethanol) are only moderately effective against the virus (Schurmann and Eggers, 1985; Mbithi *et al.*, 1993; Kramer *et al.*, 2006).

25.3.4 Avian Influenza virus

The WHO coordinates a Global Influenza Surveillance Network, with 119 National Influenza Centres in 90 countries (WHO, 2007). These centres monitor the incidence of influenza, identify circulating strains and constantly update the risk for the emergence of a pandemic strain (Stohr, 2003). The highly pathogenic H5N1 influenza virus of recent public health significance has caused a limited number of human infections worldwide, but has been circulating in poultry since 2003 with no signs of abating (ECDC, 2007). These natural infections continue to pose a potential risk to humans who interact with infected flocks or who consume raw or undercooked poultry products (Swayne, 2006b).

All influenza viruses are predominantly spread via aerosolized droplets that enter the respiratory tract of a susceptible host. Experimental models have shown that this transmission is most efficient at low temperatures and low relative humidity, in correlation with the winter seasonal peaks in influenza infections (Lowen *et al.*, 2007). The virus can persist for days when exposed to levels of solar radiation predicted for wintertime in temperate regions (Sagripanti and Lytle, 2007). The H5 strains have been shown to persist for weeks or months in water (Brown *et al.*, 2007). Although the influenza virus is enveloped, it resists drying to some extent and exhibits a 1 log reduction in 24 h on stainless steel surfaces (Noyce *et al.*, 2007).

There is also a risk that the avian influenza viruses will spread through contaminated food products. Natural infections in chickens and ducks have been demonstrated to produce contaminated meat, blood and bone (Lu *et al.*, 2003b; Swayne, 2006a; Thomas and Swayne, 2007). The efficacy of physical and chemical inactivation methods has been shown to vary with influenza virus type, and not all methods have been tested with the relevant H5N1 strain (De Benedictis *et al.*, 2007). Tables 25.3 and 25.4 summarize the information available.

The influenza viruses have a highly variable response to acid (Scholtissek, 1985a, b). The viruses are sensitive to heating, in buffer and in poultry products (Swayne and Beck, 2004; Swayne, 2006a, Thomas and Swayne, 2007). Although the highly pathogenic H5 strains have an increased heat resistance compared to low pathogenic avian influenza strains, they are inactivated at 70 °C, a temperature to which poultry is usually cooked (Swayne and Beck, 2004; Swayne, 2006a; Thomas and Swayne, 2007). High-pressure treatment at 500 MPa has also been shown to be an effective means of inactivating the viruses (Isbarn *et al.*, 2007).

Influenza viruses are enveloped, which confers susceptibility to many types

of disinfectants (De Benedictis *et al.*, 2007). Some of the agents specifically tested using avian influenza strains are listed in Table 25.4 (King, 1991; Lu *et al.*, 2003a; De Benedictis *et al.*, 2007; Suarez *et al.*, 2003).

25.4 Detection methods

25.4.1 Overview

Classical virus detection methods involve the inoculation of cell cultures to amplify and detect infectious virus particles. These methods are rarely applicable to the isolation of foodborne viruses. Many of the important viruses do not grow in cell culture and the food extracts that need to be tested may be toxic to the cells. For example, although a model system exists for the study of inactivation kinetics, wild-type isolates of the hepatitis A virus grow very slowly, if at all, in culture and accumulate many mutations during this process (Cromeans *et al.*, 1987; Konduru and Kaplan, 2006). A recently developed hepatitis E culture system takes 60 d to amplify the virus to high levels (Tanaka *et al.*, 2007). Alternative methods that rely on detection of the viral particle, such as electron microscopy and enzyme-linked immunosorbent assays, typically have detection limits on the order of 10^5 particles per gram of sample, while the infectious dose for foodborne viral infections has been estimated to be as few as 10–100 particles (Fiore, 2004; Koopmans and Duizer, 2004).

The advent of molecular methods for the detection of viral genomes has provided the increased detection sensitivity required to allow for a more accurate assessment of the viral contamination of food products (Sanchez *et al.*, 2007). Although these methods do not distinguish between infectious and non-infectious particles, their advantages are so great that they are now used for the detection of all viruses in many food virology laboratories (Jothikumar *et al.*, 2006; Sanchez *et al.*, 2007). Molecular techniques also have the advantage that they provide information on the genotype of the strains involved in outbreaks (Nainan *et al.*, 2006). This information can be useful in establishing an epidemiological link between cases of foodborne illness (Hutin *et al.*, 1999). Microarrays are also being developed that could provide genotyping without the need for sequencing amplicons (Pagotto *et al.*, 2008). The procedures outlined in the following sections describe viral extraction methods that are specific to different food types. In most cases, the extracted virus can be subsequently detected by a conventional, immunological or molecular method, as desired.

25.4.2 Detection of viruses in shellfish

Methods to extract viruses from shellfish have been extensively studied. Most methods begin by homogenizing the shellfish tissue prior to viral extraction (Sanchez *et al.*, 2007). Changing pH can be used to concentrate enteroviruses

from oysters (Sobsey *et al.*, 1975). At low pH, virus adsorbs to shellfish tissue and can be concentrated by centrifugation. At high pH, the virus is eluted from the tissue. Ultrafiltration or ultracentrifugation are then able to concentrate the viral particles in solution. The main disadvantage of this type of system is that many contaminants remain in solution with the extracted virus and may interfere with downstream detection by either cell culture or molecular methods (Speirs *et al.*, 1987). Additional concentration steps using organic flocculation or polyethylene glycol precipitation can improve the detection efficiency (Traore *et al.*, 1998). Extremely sensitive detection is obtained by lysing the viral particles with a phenol-guanidinium chloride reagent and purifying the genome using magnetic poly(dT) beads (Kingsley and Richards, 2001). This method has been standardized and published in the Health Canada Compendium of Analytical Methods for the microbiological analysis of potentially contaminated shellfish (Trottier *et al.*, 2006).

25.4.3 Detection of viruses in other foods

The development of procedures to isolate viruses from non-shellfish food samples has been more recent. Methods for viral isolation from fruits and vegetables employ washes or elution from the food surface, because homogenization releases many inhibitors of molecular reactions (Sanchez *et al.*, 2007). Methods have been developed mainly for leafy greens, green onions and berries using a variety of concentration procedures (Bidawid *et al.*, 2000c; Shan *et al.*, 2005; Guevremont *et al.*, 2006; Rzezutka *et al.*, 2006; Butot *et al.*, 2007; Papafragkou *et al.*, 2008). Polyethylene glycol precipitation (Guevremont *et al.*, 2006), immunomagnetic concentration (Bidawid *et al.*, 2000c; Shan *et al.*, 2005), charge-based concentration (Bidawid *et al.*, 2000c; Papafragkou *et al.*, 2008), ultracentrifugation (Rzezutka *et al.*, 2006) and ultrafiltration (Butot *et al.*, 2007) have all been reported to be useful for the isolation of virus particles from fruits and vegetables. For example, positively charged beads circulated and captured using the Pathatrix™ machinery yield detection limits below one plaque forming unit of the hepatitis A virus in some artificially-inoculated samples (Papafragkou *et al.*, 2008). In most cases, the choice of the method used is based on the reagents available to the testing laboratory, and the use of internal controls is not consistent. This is a concern because of the potential release of inhibitory compounds and should be addressed in future studies (Sanchez *et al.*, 2007).

25.4.4 Detection of viruses in drinking water

The detection of viruses in drinking water has traditionally involved concentration from large volumes of water using charged filters (Hill *et al.*, 1976). These methods used conventional cell culture methods to detect virus, and were thus hampered by a lack of sensitivity as well as the inability to detect non-culturable viruses. The advent of molecular methods has reduced

the time and cost required for detecting viruses from water samples (Pillai, 1997). These new methods have successfully been used to detect viral genomes or genome fragments in many types of raw and treated water samples (Kittigul *et al.*, 2000; Albinana-Gimenez *et al.*, 2006). It has, however, been demonstrated that viral genome fragments (non-viable) are detected after wastewater treatment protocols that eliminate infectious virus (Simonet and Gantzer, 2006). This raises concerns that the presence of genome fragments from enterically infecting viruses may not accurately predict the level of infectious virus in water samples.

25.5 Control issues

25.5.1 Hepatitis A virus

The sources of hepatitis A in the food supply are highly variable. The long incubation period before infection is clinically apparent and the high rate of person-to-person transmission during hepatitis A outbreaks makes point sources difficult to identify (Nainan *et al.*, 2005; Fiore *et al.*, 2006). The high proportion of asymptomatic infections in children under the age of five years generates another source of uncertainty in epidemiological investigations (Staes *et al.*, 2000). The accurate identification of disease transmission routes is a significant barrier to the implementation of effective control strategies to prevent hepatitis A virus transmission. The use of molecular detection and genotyping methods is one way to increase the odds of identifying linked cases of hepatitis A infection (see Section 25.4.1).

Due to the high degree of uncertainty in hepatitis A virus transmission, vaccination is one potential strategy for the control of infection (AAPCID, 2007). There is a safe and effective vaccine, and it is currently recommended for use in travellers to and residents of areas of high endemicity (AAPCID, 2007; CDC, 2007). Universal vaccination would be expensive, and has an unattractively high cost/benefit ratio when the healthy adult population of the developed world is the intended target (Anonychuk *et al.*, 2008). The use of sanitary measures to eliminate fecal contamination of foodstuffs should effectively limit foodborne hepatitis A outbreaks.

Pre-harvest control strategies are attractive because, when properly implemented, they minimize the need for downstream interventions. The most effective approach to prevent shellfish contamination is to prevent human sewage from entering shellfish growing waters. This sounds straightforward, but its enforcement can be difficult, particularly in remote areas with both commercial and recreational boat traffic. Imposing monetary penalties for waste dumping, mandating the use of waste containers that cannot easily be dumped overboard, and developing education outreach programs are three strategies with the potential to limit hepatitis A contamination of shellfish (Papafragkou *et al.*, 2006). If waters are contaminated, depuration can be used to reduce the levels of hepatitis A virus in shellfish prior to harvest,

but viruses in shellfish tissue are purged more slowly than bacteria, and the hepatitis A virus in particular is not as readily depurated as other viruses (Richards, 2001; Chironna *et al.*, 2002). Unfortunately, current routine testing procedures do not look for viral contaminants in growing waters, and it has been repeatedly shown that the traditional bacterial indicators are not indicative of hepatitis A virus contamination (Crocì *et al.*, 2000; Muniain-Mujika *et al.*, 2003; Pusch *et al.*, 2005; Phanuwat *et al.*, 2006; Villar *et al.*, 2007).

For the pre-harvest control of produce contamination, it is important to prevent human waste contamination of irrigation water. Although treatment regimens are available to allow the reuse of wastewater for irrigation, they are not necessarily effective against the hepatitis A virus (Gantzer *et al.*, 1998; Skraber *et al.*, 2007). Further research is necessary into the effectiveness of various water treatment protocols to determine if they are appropriate for the control of hepatitis A virus contamination. Produce is also sensitive to contamination introduced by human handling during harvest. Control procedures at this stage should include provision of toilet and hand-washing facilities, education on hygienic practices, reporting of active illnesses and provision of childcare so that young children, a prominent source of asymptomatic hepatitis A infections, are not present in the fields (Fiore, 2004; Koopmans and Duizer, 2004).

After harvest, the physical and chemical decontamination methods discussed in detail in Section 25.3.1 can be used to eliminate the hepatitis A virus from contaminated foods and/or processing areas. These are more stringent procedures than those necessary to reduce most bacterial contamination, and must be implemented properly in order to be effective. Cooking will inactivate the hepatitis A virus, but the entire product must reach 85 °C to ensure viral reduction (Parry and Mortimer, 1984). This is not suitable for fresh produce, and new technology must be developed to inactivate the virus in these products. Categories of food matrices must be individually tested to develop protocols that adequately reduce hepatitis A titre (Crocì *et al.*, 1999; Bidawid *et al.*, 2000d; Deboosere *et al.*, 2004). The use of UV light and high hydrostatic pressure are promising, but their effectiveness must be further investigated before they will be useful for routine decontamination procedures (Nuanualsuwan *et al.*, 2002; Kingsley *et al.*, 2006). Gamma irradiation is somewhat effective at reducing hepatitis A titre on lettuce and strawberries, but the dose for a 1 log reduction is approximately 3 kGy (Bidawid *et al.*, 2000b), while current regulations in the USA only allow doses up to 1 kGy for fresh foods (CFSAN, 2007).

Food handlers are another source of hepatitis A virus infections (Fiore, 2004; Greig *et al.*, 2007; Todd *et al.*, 2007a, b). Contamination may be introduced during the final preparation stages for ready-to-eat foods (Greig *et al.*, 2007; Todd *et al.*, 2007a, b). The hepatitis A virus is excreted for up to two weeks prior to the development of symptoms (Fiore, 2004). It is therefore important to stress proper hygiene and hand-washing practices for all food

service workers. Proper hand-washing with soap and water has been shown to be more effective at removing hepatitis A virus from hands than ethanol-based hand rubs (Mbithi *et al.*, 1993; Bidawid *et al.*, 2000a). Educational programs for food service workers must be designed with care to ensure the correct message is communicated. For example, gloved hands are frequently viewed as safer for food handling than bare skin, but care must still be taken to avoid cross-contamination of foods or surfaces. Preliminary data from our laboratory indicates that contaminated gloves spread virus very effectively (Bidawid *et al.*, 2007). Effective surface decontamination can also be used to interrupt hepatitis A virus transmission in food service settings, but as described in Section 25.3.1, not all commercial disinfectants are effective against the hepatitis A virus (Mbithi *et al.*, 1990; Abad *et al.*, 1997a; Bidawid *et al.*, 2000a; van Engelenburg *et al.*, 2002; Jean *et al.*, 2003; Bigliardi and Sansebastiano, 2006; Terpstra *et al.*, 2007). For effective disinfectants, such as sodium hypochlorite, concentration and contact time must be followed precisely to effectively inactivate the virus on a contaminated surface (Grabow *et al.*, 1983; Mbithi *et al.*, 1990; Abad *et al.*, 1997a; Jean *et al.*, 2003).

25.5.2 Hepatitis E virus

The control of hepatitis E infections in developing countries can be achieved by improving the availability of clean drinking water. This is linked to the availability of adequate hygienic facilities and improved hygienic practices. There is no vaccine available against hepatitis E, and the administration of pooled immunoglobulin from endemic areas does not appear to be protective (Khuroo and Dar, 1992; Panda *et al.*, 2007). Since it has been shown that the virus can survive the levels of chlorination currently recommended by the WHO, research into the physical and chemical inactivation of hepatitis E is urgently required to provide protocols that ensure the disinfection of contaminated water supplies (Guthmann *et al.*, 2006). Experimental studies in pig livers and epidemiological evidence indicate that boiling water is sufficient to inactivate the virus (Velazquez *et al.*, 1990; Feagins *et al.*, 2008).

As an emerging foodborne zoonotic agent, there is little information available about control measures that will prevent the spread of hepatitis E infection. The infection is endemic in many swine populations that have been examined, but does not cause overt disease. As a result, there is no incentive for control measures to improve animal health (Goens and Perdue, 2004). If the link between infected animals and transmission to humans can be established outside of Japan, this might provide a rationale for the development of animal-specific prevention strategies. At this time, however, the most effective control measure against infection is thorough cooking of meats and organ meats prior to consumption (Feagins *et al.*, 2008). Because of the low rates of person-to-person transmission in documented outbreaks, transmission via food handlers and ready-to-eat foods is not expected to be a major source of infection (Hla *et al.*, 1985; Somani *et al.*, 2003).

25.5.3 Poliovirus

Control measures against poliovirus involve vaccination programs and the global eradication initiative (Arya and Agarwal, 2007; Chumakov *et al.*, 2007). The virus does not have a non-human host, and it cannot circulate if the human population has a high level of mucosal immunity to infection (Melnick, 1996). Unfortunately, in addition to the four remaining endemic countries, 21 countries have experienced a resurgence or importation of poliomyelitis in recent years (Lahariya, 2007). This is due to the high prevalence of asymptomatic infections, as well as to vaccine-derived strains causing disease in communities (Chumakov *et al.*, 2007; Lahariya, 2007).

Fortunately, some relatively straightforward measures can be implemented to ensure that the poliovirus, if circulating, does not enter the food supply. Pasteurization of milk products has been shown historically to disrupt poliovirus transmission (Sattar *et al.*, 2001). Similar time/temperature combinations (72 °C, 30 s) can be used to inactivate the virus in other potentially contaminated liquids (Strazynski *et al.*, 2002). Depuration of shellfish greatly reduces the risk of poliovirus transmission by this route, and proper hand-washing by food handlers interrupts the chain of transmission during final preparation of foods (Schurmann and Eggers, 1985; Franco *et al.*, 1990; Enriquez *et al.*, 1992). It should be noted that for all of these measures, the conditions required to eliminate polio are less stringent than those required for the inactivation of hepatitis A (Table 25.3).

The presence of the poliovirus in wastewater remains the most important means of transmission in endemic countries, and effective methods exist to remove polio from sewage (Pavlov, 2006; Arraj *et al.*, 2005; Belguith *et al.*, 2007; Dedepsidis *et al.*, 2007). New methods under development, such as ozone and UV light, are able to decontaminate poliovirus-contaminated wastewater (Lazarova and Savoys, 2004; Tanner *et al.*, 2004). The required focus on sewage treatment and clean water in the developing world is reminiscent of control measures to prevent the spread of many bacterial illnesses (Berry *et al.*, 2006). The integration of clean water programs aimed at reducing the burden of bacterial and viral illness can only serve to increase the likelihood that these programs will see some successes in limiting the spread of enteric disease.

25.5.4 Avian Influenza virus

Current strategies to mitigate the human health risks associated with the H5N1 avian influenza virus are mainly focused on preventing the disease from spreading in the animal population (Rajagopal and Treanor, 2007). The elimination of H5N1 infections in birds would of course remove the risk to humans. Unfortunately, H5N1 infections in birds have become endemic in many countries (ECDC, 2007, Rajagopal and Treanor, 2007). Therefore, accurate monitoring and understanding the circulation of the virus in wild and domestic birds is critical to the success of control programs (Olsen *et*

al., 2006). All H5 or H7 type avian influenza infections are notifiable to the World Organization for Animal Health (OIE, 2007). Early detection of the H5N1 infection in a local poultry population is a key step in preventing the disease from becoming widespread (Sims, 2007).

A vaccine is available against the H5 and H7 avian influenza virus subtypes, and its use in the poultry population is one way to control the spread of emerging highly pathogenic viruses (Capua and Marangon, 2007). It is not possible to vaccinate all birds in areas where these viruses are endemic, and additional measures must be in place to prevent the spread of disease (Guan *et al.*, 2007; Sims, 2007). A combination of surveillance, vaccination, culling of infected birds and segregation of wild and domestic poultry is recommended to control the spread of emerging epidemic avian influenza strains (Guan *et al.*, 2007).

Control measures to prevent transmission of avian influenza through the food supply are more straightforward. All of the strains and sub-types of influenza are more susceptible to heating and to chemical disinfection than the other foodborne viruses described in this chapter (see Tables 25.3 and 25.4). Thorough cooking of poultry products and basic disinfection of food preparation surfaces is sufficient to prevent foodborne transmission of the influenza virus (De Benedictis *et al.*, 2007).

25.6 Future trends

Two of the four viruses discussed in detail in this chapter are emerging zoonoses (hepatitis E and avian influenza). It is important that public health programs continue to monitor these diseases in both animals and humans in order to develop accurate risk assessment and prevention planning (Merianos, 2007). The other two viruses (hepatitis A and polio) cause human illnesses that are vaccine-preventable. Calls from experts to continue and expand vaccine coverage with the goal of reducing the disease burden from these viruses is likely to continue (AAPCID, 2007; Chumakov *et al.*, 2007).

In addition to public health measures, the food production and processing industries can take action to reduce the spread of viruses through food. A recurring point in the above discussion is the remarkable resistance of viruses to decontamination procedures. Heating and disinfection protocols for viruses and food preparation surfaces are being defined in the literature, but they must also be recognized and implemented along the food production continuum. In addition, these viruses typically have a very low infectious dose (10–100 particles) and they can be excreted at high levels (10^6 – 10^{11} particles per gram of faeces). Since contamination of food products is typically a secondary event, a 5 log reduction in infectious particles has been considered effective for control. The recent development of sensitive and semi-quantitative detection methods will help to identify the critical control points along the food production and preparation continuum where

virus contamination can be reduced. Unfortunately, many of these methods detect viral genomes instead of infectious virus particles. The regulation of viruses in foods will require a more detailed understanding of the correlation between the presence of viral nucleic acid fragments and a human health risk.

25.7 Sources of further information and advice

A book dedicated to food virology:

Goyal S M and Doyle M P (2006) *Viruses in foods*, New York, Springer. Comprehensive reviews of the four viruses discussed in detail in this chapter:

Abdel-Ghafar A N, Chotpitayasunondh T, Gao Z, Hayden F G, Nguyen D H, De Jong M D, Naghdaliyev A, Peiris J S, Shindo N, Soeroso S and Uyeki T M (2008) Update on avian influenza A (H5N1) virus infection in humans, *N Engl J Med*, **358**, 261–73.

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