Enteric hepatitis viruses

Description

The term “hepatitis viruses” refers to a diverse group of viruses all of which have the human liver as the primary target of replication and give rise to hepatitis or inflammation of the liver. Their replication may result in mass destruction of liver cells. Consequences include failure of the liver to fulfil basic functions such as removal of bilirubin from the circulatory blood system. Bilirubin is a red pigment released from red blood cells as they break down and are replaced by new cells. Excessive accumulation of the pigment in the bloodstream is manifest as yellow coloration of sites such as the eyes and the palms of the hands. This condition is known as jaundice and is also marked by dark urine and stools resulting from the excretion of bilirubin. Another typical consequence of massive liver cell damage is release into the bloodstream of liver enzymes, including alanine transaminase (ALT) and aspartate transaminase (AST). Serum levels of these enzymes are used to diagnose hepatitis (Zuckerman & Thomas, 1993).

Hepatitis may also be caused by other systemic pathogens such as cytomegalovirus, yellow fever virus, and *Leptospira* bacteria, although the liver is not the primary or only target of these organisms. Liver cell damage and jaundice may also be caused by toxic compounds, including alcohol.

Since the clinical symptoms caused by hepatitis viruses are very similar, and some of the viruses only emerged on a large scale in recent years, distinction of different aetiological agents has been progressively accomplished only since the 1960s. The first two hepatitis viruses that were distinguished were simply

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designated A and B, because at that time there was no indication of more. As new hepatitis viruses were discovered, the alphabetical nomenclature was retained. The range has already reached G, and there are indications of more hepatitis viruses. Unfortunately, this non-descriptive system of alphabetical nomenclature is confusing to the non-expert in the field. The nomenclature is abbreviated as HAV to HGV for hepatitis A to G viruses.

Hepatitis viruses are divided into two basic groups, some distinctive features of which are summarized in Table 4.

The group referred to as enteric hepatitis viruses consists of HAV, HEV, and HFV. The parenterally transmitted or bloodborne hepatitis viruses form the second group and consist of HBV, HCV, HDV, and HGV. Enteric hepatitis viruses are transmitted primarily by the faecal–oral route: explosive epidemics of HAV, and particularly of HEV, usually result from faecal contamination of water or food. The parenterally transmitted viruses are transmitted primarily by blood and blood products—by medical transfusion, as well as by sexual intercourse, use of contaminated medical instruments such as syringes and needles, and even by tattooing and insect bites. There is no evidence that parenterally transmitted viruses are of significant concern to water quality. HBV seems to be inactivated

### Table 4. Classification of hepatitis viruses

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<thead>
<tr>
<th>Hepatitis virus</th>
<th>Characteristic featuresa</th>
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<tr>
<td><strong>Enteric</strong></td>
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<tr>
<td>Hepatitis A (HAV)</td>
<td>Family Picornaviridae; non-enveloped 27–32 nm icosahedral particles; ssRNA; no chronic infection or carrier state; vaccines available; worldwide; low mortality</td>
</tr>
<tr>
<td>Hepatitis E (HEV)</td>
<td>Family Caliciviridae; non-enveloped 25–35 nm icosahedral particles; ssRNA; no chronic infection or carrier state; no vaccine available; restricted geography; high mortality in pregnant women</td>
</tr>
<tr>
<td>Hepatitis F (HFV)</td>
<td>Unclassified; non-enveloped 27–37 nm icosahedral particles; dsDNA; no chronic infection or carrier state; no vaccine available; apparently restricted geography and low mortality</td>
</tr>
<tr>
<td><strong>Parenterally transmitted</strong></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B (HBV)</td>
<td>Family Hepadnaviridae; enveloped 42 nm spherical particles; dsDNA; sometimes chronic carrier state; vaccines available; worldwide; sometimes leads to fatal liver cancer</td>
</tr>
<tr>
<td>Hepatitis C (HCV)</td>
<td>Family Flaviviridae; enveloped 50 nm spherical particles; ssRNA; chronic infection in &gt;50% of cases; no vaccine available; worldwide</td>
</tr>
<tr>
<td>Hepatitis D (HDV)</td>
<td>Classified as sub-viral agent; enveloped 35–37 nm spherical particles; ssRNA; often chronic infection; no specific vaccines; restricted geography; defective virus dependent on HBV</td>
</tr>
<tr>
<td>Hepatitis G (HGV)</td>
<td>Family Flaviviridae; enveloped 35–37 nm spherical particles; ssRNA; often chronic infection; no vaccine available; worldwide</td>
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a ss = single-strand; ds = double-strand.
by enzymes produced by bacteria in the gastrointestinal tract and water resources (Grabow et al., 1975), and infectious HBV is therefore rarely, if ever, detectable in faeces, water, or food. This seems also to be true of HCV, HDV, and HGV, and so there is no further discussion of these viruses here.

The global public health impact of enteric hepatitis virus infections is immense. No attempt has yet been made to calculate meaningful figures because of the effect of variables such as the relationship between infections and standard of living, subclinical cases, and under-reporting. However, enough data exist to give an indication of general trends. For instance, statistics for the United States indicate that viral hepatitis was the second most frequently reported infection in 1990 after sexually transmitted diseases (Zuckerman & Thomas, 1993). In 1981, 57,929 cases of viral hepatitis (25.3/100,000 population) were reported, of which 45% were hepatitis A. Records show 31,441 cases of hepatitis A (12.64/100,000 population) in 1990 and 31,582 cases in 1995 (American Academy of Pediatrics Committee on Infectious Diseases, 1996). The direct and indirect cost per clinical case of viral hepatitis A infection has been estimated at US$ 1000 for individuals aged up to 18 years and US$ 2100 for those older than 18 years. A study of a foodborne outbreak of hepatitis A in Denver, Colorado, in 1992 with 43 secondary cases and potential exposure of approximately 5000 people, revealed direct medical costs associated with infected patients of US$ 46,064. This was only 7% of the US$ 689,314 spent on controlling the disease (epidemiological studies to locate the source of the outbreak, interventions to prevent infection of potentially exposed individuals, and related expenses) (Dalton et al., 1996). Extrapolation of these cost estimates to episodes such as the 1988 shellfish-associated outbreak in Shanghai, China, with close to 300,000 clinical cases of hepatitis A and 32 deaths (attack rate 4083/100,000 population), yields dramatic figures. The United States cost figures for hepatitis A are likely to be representative of developed countries. Hepatitis A morbidity patterns are different for developing countries, however, where subclinical infection in childhood years may be almost universal (Zuckerman & Thomas, 1993). Although no corresponding cost estimates have yet been reported for viral hepatitis E, the costs may be even higher than for hepatitis A, since the infection is generally contracted later in life, with a relatively high incidence of clinical cases and mortality in pregnant women. The financial burden of hepatitis E in countries where it is the most common cause of viral hepatitis (Grabow, 1997) is therefore likely to be extremely high. Likewise, the public health burden of single outbreaks, such as those with more than 100,000 cases in China between 1986 and 1988, and an estimated 79,000 cases in India in 1991 (Grabow, 1997), is enormous.

**Hepatitis A virus**

Of the three enteric hepatitis viruses currently recognized, HAV has the longest and best known history. Initially HAV was known as the “infectious” or “epidemic” hepatitis virus, because of its typical association with epidemics caused
by contaminated water and food worldwide (Mosley, 1959; Grabow, 1976; Zuckerman, 1983). Extreme examples include the 1988 outbreak of some 300,000 cases in Shanghai, China, caused by the consumption of clams harvested from a bay polluted with sewage from a community that had experienced an epidemic of hepatitis A (Halliday et al., 1991).

HAV is a typical member of the family Picornaviridae. It has a non-enveloped icosahedral capsid of diameter 25–35 nm and a single-strand RNA genome. HAV shares many features with members of the genus Enterovirus, such as polioviruses and coxsackieviruses, and has at some time been classified as enterovirus type 72. More recently, however, HAV has been classified in its own genus, known first as Heparnavirus and then as Hepatovirus.

Initial multiplication of picornaviruses like polioviruses, coxsackieviruses, and HAV takes place in the lymphoid tissue of the pharynx, and these viruses are detectable in throat swabs and sputum specimens during early stages of infection. The predominant site of replication is the lymphoid tissue of the gut, and the viruses are therefore typically detectable in stool specimens. Only in a small percentage of cases do enteroviruses proceed to infect the central nervous system, and HAV to infect the liver. HAV replication in the liver causes damage to liver cells, which is known as hepatitis. In immune individuals, however, circulating antibodies prevent HAV from infecting the liver.

Like many picornaviruses, HAV tends to cause infections that are mild or without clinical symptoms in children. The incidence of infection is closely linked with hygiene and sanitation conditions, and most people in developing countries contract infections during early childhood. Typical clinical symptoms of infection are predominantly seen in adults. Although mortality is generally less than 1%, the disease may be quite severe and incapacitating; there may be substantial liver damage, and regeneration of the liver cells takes time (Zuckerman & Thomas, 1993). Patients may feel ill and be confined to bed for up to 6 weeks or more; they usually lack interest in foods that depend heavily on liver functions for digestion. The severity of illness and mortality may be dependent on underlying conditions such as immunodeficiencies and malnutrition, and on the general state of health.

There seems to be only one antigenic type of HAV, which elicits lifelong immunity. In adult populations of developing countries and communities, immunity to HAV may exceed 95%, in contrast to developed countries and communities where levels of immunity may be less than 50% (Iwarson, 1992; Sathar et al., 1994; Tucker et al., 1996). People from developed countries who visit developing areas are therefore exposed to a high risk of infection. HAV typically occurs in all parts of the world and, beyond the link to standards of hygiene and sanitation, gives no indication of geographical preferences.

Unlike closely related viruses such as polioviruses and some coxsackieviruses, HAV is not readily detectable by routine cell culture procedures. Many questions about the epidemiology of the virus, as well as about its occurrence and behaviour in the environment, therefore remain unanswered. However, there is
little doubt that the virus is highly infectious and can cause explosive outbreaks when present in water or food. In addition, it is relatively resistant to unfavourable environmental conditions, including water treatment and disinfection processes. High infectivity has been demonstrated for closely related viruses such as echoviruses and poliovirus vaccine strains in experiments using human volunteers (Grabow, 1996). In terms of its transmission by water and food, HAV is even more infectious, as shown by the high risk of infection associated with faecally polluted water and food—even supplies that meet generally accepted quality limits for coliform indicator bacteria (Bosch et al., 1991a, 1991b). Closely related viruses such as polioviruses, coxsackieviruses, and echoviruses are less frequently associated with waterborne transmission (Grabow, 1996).

Much of the available information on HAV derives from experiments with human volunteers. Well-known studies include those of Neefe et al. (1947) carried out during the Second World War in attempts to control a disease that is notorious for its devastating impact on troops and civilians alike during times of war. This work was followed some years later by experiments on another group of human volunteers, which resulted, among other things, in the first distinction between HAV and HBV (Krugman & Giles, 1970).

An important step forward was the visualization of the virus by immune electron microscopy, and then propagation of the virus in certain primates, notably marmosets and chimpanzees (see Grabow et al., 1981). This led to the development of immunological assays for detection of the virus and its antibodies (Coulepis et al., 1985). Another milestone was the discovery that HAV can replicate slowly in certain cell cultures (Frösner et al., 1979), and this led to the establishment of cell-culture-adapted strains (Gust et al., 1985). Although these adapted strains may differ in some respects from wild-type HAV, they made it possible to study the behaviour of the virus in the environment and to develop vaccines, which are now freely available. The advent of molecular techniques, notably gene probe hybridization and the polymerase chain reaction (PCR), led to the development of sensitive techniques for detection of the virus (Dubrou et al., 1991; Deng, Day & Cliver, 1994; Tsai et al., 1994).

The incubation period of hepatitis A may vary from 15 to 45 days, with a mean of 30 days (Reid & Dienstag, 1997), i.e. some 10 days less than the incubation period of hepatitis E. Faecal excretion of HAV begins late in the incubation period, peaks just before onset of clinical symptoms of disease (usually the appearance of dark urine), and falls to barely detectable levels as the clinical illness evolves. The virus is present in blood in relatively low numbers for about 7–14 days, with a peak before the onset of clinical symptoms (Zuckerman & Thomas, 1993).

Typically, faecal–oral transmission of HAV is the result of personal contact or the consumption of faecally polluted water or food. A common sequence of events is for one member of a household to contract the infection from contaminated water or food, or from contact with an infected individual outside the home, and then to infect other members of the household by personal contact.
Household infection rates of up to 95% have been recorded (Villarejos et al., 1982). This secondary transmission is difficult to predict or prevent because the virus is transmitted within the family before infection of the primary case is evident (Halliday et al., 1991; Zuckerman & Thomas, 1993). Although a variety of non-human primates are susceptible to HAV under experimental laboratory conditions, and transmission of the virus from chimpanzees to humans during close contact is well documented (Grabow et al., 1981; Zuckerman & Thomas, 1993), there is no evidence that animals may serve as a significant reservoir for HAV.

Hepatitis E virus

The existence of HEV was confirmed in the late 1970s and early 1980s (Wong et al., 1980), after it became evident that there was a hepatitis virus other than HAV and HBV. Infection with the virus was initially referred to as enterically transmitted (or epidemic) non-A, non-B hepatitis. It eventually transpired that HEV had for many years been mistaken for HAV, because the two viruses share certain basic clinical and epidemiological properties (Grabow et al., 1994; Purcell, 1996). Both are transmitted primarily by the faecal–oral route, and are often associated with waterborne and foodborne outbreaks. However, viral hepatitis E tends to occur more often in young adults, many of whom are already immune to hepatitis A (Purcell, 1996). In contrast to hepatitis A, which rarely causes complications, hepatitis E tends to give rise to more prominent cholestasis and the infection can present as acute fulminating hepatitis, particularly in pregnant women, for whom case fatality rates as high as 20–40% have been recorded. Hepatitis E has an incubation period of 14–16 days, with a mean of 40 days (Reid & Dienstag, 1997), which is longer than that of hepatitis A. An exceptionally long viraemia is typical for HEV infection, generally lasting for as long as 6 weeks, and in some cases up to 16 weeks—again, substantially longer than for HAV. Patients generally excrete HEV for 1–2 weeks; in one case, however, the virus was excreted for more than 7 weeks, well after clinical and biochemical recovery (Clayson et al., 1995; Scharschmidt, 1995; Purcell, 1996).

Secondary transmission of HEV from cases to contacts has been reported but appears to be much less common than is true of HAV (Purcell, 1996). This seems surprising given that the duration of both faecal excretion and viraemia is substantially longer for HEV than for HAV. The low level of person-to-person spread probably implies that faecally polluted water plays a much more important role in the spread of HEV than of HAV. The explosive outbreaks of hepatitis E typically associated with waterborne transmission resemble the epidemiology of hepatitis A, and suggest that HEV may be as infective as HAV. The important role of water in the transmission of HEV may allow the virus to be transmitted from animals to humans via water resources polluted with animal wastes.
Although HEV is a single-stranded RNA virus with non-enveloped icosahedral capsid similar to that of HAV, the two viruses differ substantially at molecular level and HEV has been classified as a member of the family Caliciviridae.

There are indications of antigenic variation and possibly even differences in serotypes of HEV (Chauhan et al., 1994; Purcell, 1996; Schlauder et al., 1999; Tsarev et al., 1999). These strain variations seem to have implications for the molecular detection of the virus and for serological antibody assays (Mast et al., 1996; Ghabrah et al., 1998; Webber et al., 1998), and may even affect the immune status of patients. However, no individual has yet been reported as having contracted HEV infection more than once, which suggests that infection at any age generally results in lifelong immunity, as for HAV.

Despite some resemblances between HEV and HAV, there are also marked differences in their epidemiology. Both infections are primarily associated with poor standards of hygiene and sanitation, but the epidemiology of HEV seems also to include a geographical element (Grabow, Taylor & Webber, 1996). Clinical infections and outbreaks of hepatitis E have been recorded predominantly in countries such as Afghanistan, China, India, Myanmar, Nepal, and Pakistan, on the island of Borneo, and in parts of Central Asia; in Mexico; and in parts of Africa such as Algeria, Côte d’Ivoire, Egypt, Ethiopia, Somalia, and Sudan. The disease is endemic in many of these countries, and is the most common cause of acute hepatitis in adults in parts of India, other parts of Asia, and in Africa. Large outbreaks associated with sewage-contaminated drinking-water include one in 1954 involving approximately 40,000 cases in Delhi, India, one in 1986–1988 with more than 100,000 cases in the Xinjiang Uighar region of China, and one in 1991 with some 79,000 cases in Kanpur, India (Grabow et al., 1994; Scharschmidt, 1995).

Clinical cases, and outbreaks particularly, seem to occur rarely in parts of the world such as Japan, South Africa, the United Kingdom, North and South America, Australasia, and central Europe (Craske, 1992; Grabow, Taylor & Webber, 1996). Most cases that do occur in these parts of the world are imported. However, seroprevalence studies now reveal that the virus is actually present in many of these countries, and some 2–10% of the population may have antibodies, confirming exposure to the virus. Why there should be a relatively low incidence of clinical cases and outbreaks in certain parts of the world, despite the presence of the virus, is not yet fully understood (Grabow, Taylor & Webber, 1996). Answers to this and related questions are of fundamental importance because they may hold the key to methods for preventing worldwide spread of the virus and for control of the disease (Scharschmidt, 1995).

Since HEV is not readily detectable by conventional cell culture procedures, most of the initial work on the virus was confined to studies involving human volunteers, electron microscopy, immunological assays, and epidemiological data. The discovery that HEV causes infection in certain primates that resembles the infection in humans cast new light on the virus and its epidemiology (Bradley...
et al., 1987). Research progress accelerated when molecular techniques became available (Favorov et al., 1992; Jothikumar et al., 1993). Subsequent studies revealed that at least some strains of the virus may also replicate in a variety of other animals, including laboratory rats (Maneerat et al., 1996; Meng, Guinet & Pillot, 1996), domestic pigs (Balayan et al., 1990), and rhesus monkeys (Sharma et al., 1990; Nanda et al., 1994).

The zoonotic nature of HEV was confirmed by Clayson et al. (1996) who detected the virus in a variety of wild and domestic animals (including cows, pigs, and goats); more recent findings even indicate the presence of HEV in rodents. In endemic areas the incidence of HEV in animals appears to correlate with that in humans. These findings suggest that animals may serve as a reservoir for HEV, and that many human infections may originate from water sources polluted by animal wastes (Kabrane-Lazizi et al., 1999; Wu et al., 2000). They also seem to be in agreement with the low level of human-to-human transmission mentioned earlier, and the detection of HEV in sewage in Barcelona, Spain—a part of the world where HEV is not endemic (Pina et al., 1998).

HEV thus seems to be unique as the only typical zoonotic member of the group of enteric viruses that are predominantly host-specific viruses infecting either humans or animals. If domestic and wild animals do indeed play an important role in the waterborne transmission of HEV, it underlines the need to protect water resources and supplies from pollution by animal wastes. In the past animal wastes were not considered to be of particular importance with regard to viruses: generally speaking, enteric viruses tend to be host-specific and there is scant evidence of human viral infections being contracted from water contaminated by animal wastes (Grabow, 1996).

Hepatitis F virus

The existence of HFV has not yet been conclusively proven: the name was proposed following reports of hepatitis cases associated with waterborne transmission of a virus distinguishable from HAV and HEV (Craske, 1992). Indications are that HFV is associated with sporadic cases in certain geographical areas, and not with outbreaks or epidemics (Sharma et al., 1990; Deka, Sharma & Mukerjee, 1994). Cases have been recorded in India, Italy, the United Kingdom, and the USA. In some of these areas clinical cases of HEV are virtually unknown and HAV is rare. Reasons for the apparent epidemiological pattern of HFV infection are not clear.

The virus seems to consist of a non-enveloped icosahedral particle, diameter 27–37 nm, containing double-stranded DNA. It has not yet been classified. Infection of rhesus monkeys has been reported, as has replication with cytopathogenic effect in the Hep-2 (human larynx carcinoma) cell line. Further details are primarily based on epidemiological data, electron microscopic detection of virus-like particles in patient stools, and clinical symptoms typical of enteric viral hepatitis in the absence of other causes of the disease. HFV does not
seem to be detectable by conventional routine cell culture techniques. Many questions about the virus remain to be answered, including those of infectivity, survival in the environment, and removal or inactivation by water treatment and disinfection processes.

**Monitoring and assessment**

Hepatitis viruses share the important characteristic of not readily causing a cytopathogenic effect in currently available cell culture systems. They are thus undetectable by conventional cell culture propagation procedures used for reoviruses, polioviruses, and some coxsackieviruses (Grabow et al., 1999). Well-established technology and expertise for direct monitoring and assessment of enteric hepatitis viruses in water and food are limited to HAV (Bosch et al., 1991a, 1991b; Hall & Sobsey, 1993; Tsai et al., 1994; Sobsey, Hall & Hazard, 1995). Detection of HEV in water or food has been reported only once using molecular techniques (Jothikumar et al., 1993), and there are no reports of HFV detection. Pina et al. (1998) amplified an HEV isolate from sewage in cynomolgus monkeys, but this technique would not be suitable for routine purposes. Observations that HEV may replicate in some cell cultures (Huang et al., 1992, 1995; Meng, Guinet & Pillot, 1996; Tam et al., 1996) could lead to the development of practical monitoring procedures, similar to those used for HAV, in which cell cultures are used to amplify at least the nucleic acid of the virus, which is then detected by molecular techniques (Grabow, 1997).

At this stage, even the technology for HAV is beyond the reach of many laboratories involved in water quality monitoring. Consequently, tests for hepatitis viruses are not currently recommended for conventional routine water quality monitoring. Analysis for hepatitis viruses is likely to be restricted largely to research purposes (Bosch et al., 1991a, 1991b; Grabow, 1997; Pina et al., 1998) until more practical and economical methods become available.

Assessment of the safety of water supplies with regard to hepatitis viruses—and many other viruses and other pathogens—therefore continues to depend largely upon indirect methods, such as the use of microbial indicators of faecal pollution. There is no doubt, however, that the commonly used methods have certain shortcomings with regard to indicating the presence of enteric viruses. For instance, outbreaks of hepatitis A have been associated with water supplies that conformed to generally accepted guidelines for indicators and treatment procedures (Hejkal et al., 1982; Bosch et al., 1991b; Grabow, 1997). Moreover, laboratory experiments have demonstrated that HAV, as well as at least some of the other enteric viruses, is more resistant to unfavourable conditions, including water treatment and disinfection processes, than commonly used indicators such as coliform bacteria (Grabow, 1997).

Water quality indicators should therefore be used with caution, and it may be necessary to employ combinations of indicators appropriately selected for various purposes (Grabow, 1996). Such combinations may have to include...
indicators such as phages and *Clostridium perfringens*. It may also be advisable to supplement quality monitoring and assessment by meticulous sanitary surveys, and to base strategies on specifications for the quality of raw water sources and the efficiency of treatment and disinfection processes (Lloyd & Bartram, 1991; Regli et al., 1991; Sobsey et al., 1993; States & Sykora, 1995; Grabow, 1996; World Health Organization, 1997).

Indications are that HAV can successfully be recovered from water and food using techniques commonly applied to, for example, polioviruses and reoviruses. These include adsorption–elution procedures using positively or negatively charged membrane filters, followed by organic flocculation for secondary concentration (Sobsey, Oglesbee & Wait, 1985). HAV has been recovered from seeded drinking-water samples by means of ultrafiltration at an efficiency of recovery (EOR) of 100%, higher than that for polioviruses (Divizia, Santi & Pana, 1989). In a comparison of a number of recovery techniques for HAV, Bosch et al. (1991a) obtained the best results by adsorption–elution using glass powder of which the electrostatic charge had been changed to positive by treatment with polyethylenimine. For HAV in seeded 20-litre samples EOR was 100% for tap water, 94% for seawater and 61% for fresh water and sewage. HAV has also been recovered from sewage sludge (Graff, Ticehurst & Flehmig, 1993), shellfish meat suspensions (Deng, Day & Cliver, 1994; Jaykus, de Leon & Sobsey, 1996) and drinking-water supplies (Schwab, de Leon & Sobsey, 1996) by means of antigen capture techniques, using HAV-specific antibodies to recover the virus, but no specific details on EOR are available.

Jothikumar et al. (1993) successfully recovered HEV from raw and treated sewage by means of membrane filter adsorption–elution, followed by magnesium chloride precipitation. This procedure yielded a high EOR for enteroviruses, but the EOR for HEV has not been established. Sewage samples adjusted to pH 5.0 yielded positive PCR results for HEV, but samples adjusted to pH 3.5 failed to do so, which suggests that HEV is more sensitive to low pH levels than poliovirus, for example, which is recovered at this pH level in some routine techniques. Pina et al. (1998) were also successful in recovering HEV from sewage sample using ultracentrifugation of 40-ml samples; previously, the technique had achieved 70% recovery of seeded polioviruses.

Most of the above experiments on recovery of HAV were carried out using cell-culture-adapted strains of the virus, which can be titrated by conventional cell culture procedures or plaque assays. Detection of wild type HAV, however, requires different strategies, and the most feasible current approach is based on molecular techniques. HAV has been successfully detected in samples of wastewater, river water, and treated water by direct gene probe hybridization (Jiang et al., 1986; Dubrou et al., 1991) or PCR (Goswami, Koch & Cebula, 1993; Graff, Ticehurst & Flehmig, 1993; Tsai et al., 1993; Jaykus, de Leon & Sobsey, 1996; Schwab, de Leon & Sobsey, 1996). Goswami, Koch & Sebula (1993) claimed levels of sensitivity of 10 HAV RNA molecules in a reaction mixture of shellfish meat homogenate. Tsai et al. (1993) found their reverse transcriptase
RT) PCR technique to be at least 500 times more sensitive for poliovirus than conventional cell culture detection, which suggests that the same procedure would also be extremely sensitive for HAV. In 1994 Tsai et al. reported the development of a triplex RT–PCR that simultaneously detected poliovirus, HAV, and rotavirus, radically reducing the time, cost, and labour involved in the monitoring of water supplies. Supplementation of molecular techniques by prior cell culture amplification of viral RNA substantially increases detection sensitivity, in addition to providing evidence that the detected HAV is viable (Dubrou et al., 1991; Shieh et al., 1991; Grabow, 1997).

Using a PCR procedure based on preparing HEV-specific cDNA by reverse transcription for amplification by PCR and detection by slot blot hybridization, Jothikumar et al. (1993) detected HEV recovered from sewage. The method followed by Pina et al. (1998) was similar, except that PCR products were analysed by agarose gel electrophoresis with ethidium bromide as the stain. Nested PCR products were characterized by determining nucleotide sequences (using an automated sequencer) and comparing them with HEV sequences in the GenBank and European Molecular Biology Library databases.

Information on the role of water and food in the transmission of HEV and HFV is primarily based on epidemiological data because practical methods for detection of these viruses are not yet available. Epidemiological data include indirect evidence obtained by seroprevalence studies: the presence of specific antibodies in individuals proves exposure to the virus, and a higher incidence of antibodies in communities exposed to contaminated water and inadequate sanitation indicates transmission by water and food (Grabow, Taylor & Webber, 1996; Tucker et al., 1996).

**Control**

The production of water supplies free of enteric hepatitis viruses, or with virus levels within tolerable limits, is possible and feasible yet is not always accomplished. The reasons for failure include cost, unavailability of expertise and facilities, and factors such as human negligence and error. Other contributing factors are the variable occurrence of viruses in polluted water sources (Halliday et al., 1991), and the exceptional resistance and high infectivity of the viruses—at least of HAV. Epidemiological statistics reveal that enteric hepatitis virus infections are much more frequently associated with waterborne transmission than infections with other enteric viruses (e.g. poliovirus, coxsackievirus, echovirus, adenovirus) (Grabow, 1997). In the laboratory HAV has been shown to be more resistant than faecal bacteria and certain phages to disinfecting agents such as chlorine, ozone, and hydrogen peroxide (Grabow et al., 1983; Rao et al., 1988; Mbithi, Springthorpe & Sattar, 1990; Hall & Sobsey 1993; Nasser et al., 1995). These findings are in agreement with reports on the detection of HAV in water that met generally accepted limits for faecal bacteria (Ford & Colwell, 1996; Grabow, 1996). The behaviour of HAV in the environment also differs from that of other
viruses and phages in respects such as survival in seawater and shellfish, resistance to ultraviolet light, and removal by filtration through soil columns (which reflects differences in adsorption properties) (Tsai et al., 1993; Callahan, Taylor & Sobsey, 1995; Lévêque et al., 1995; Sobsey, Hall & Hazard, 1995). The reasons for the successful transmission of enteric hepatitis viruses by water are neither fully understood nor quantifiable, and the roles of viral resistance and infectivity and of host susceptibility remain to be elucidated.

The only direct clue to the behaviour of HEV in the water environment is provided by detection of the virus in raw and treated wastewater, which suggests that the virus survives at least some wastewater treatment processes (Jothikumar et al., 1993). No such details are available on HFV. However, epidemiological evidence on their transmission by water and food leaves no doubt that all enteric hepatitis viruses must survive environmental conditions well enough to cause massive outbreaks of hepatitis A and E and sporadic case of hepatitis F.

The removal and inactivation of hepatitis viruses by individual treatment and disinfection processes commonly used in the preparation of drinking-water supplies have not yet been fully investigated. The principal reason for this is the lack of practical techniques for detecting viable naturally occurring hepatitis viruses. However, the behaviour of hepatitis viruses seems to resemble that of other viruses and phages sufficiently for data on polioviruses and reoviruses, for example, and on phages like somatic and F-RNA coliphages to provide at least an indication of what can be expected for hepatitis viruses.

In laboratory experiments on flocculation-enhanced rapid sand filtration, for instance, Nasser et al. (1995) found turbidity to be reduced by 99%, a vaccine strain of poliovirus by 80%, and a cell-culture-adapted strain of HAV and F-RNA phage MS2 both by 93%. Vaccine poliovirus was reduced by as much as 99% in units using aluminium sulfate, ferric sulfate, or ferric chloride coagulation and rapid sand filtration (Bitton, 1980). In a plant for the direct reclamation of drinking-water from wastewater, ferric chloride clarification followed by rapid sand filtration reduced naturally occurring cytopathogenic viruses by 88–99%, depending on the operating conditions (Grabow, 1990). Water-softening using a lime–soda ash process reduced vaccine poliovirus by more than 99% (Bitton, 1980). In laboratory experiments, activated carbon filters removed 75–82% of vaccine poliovirus and 53–86% of coliphage T4, depending on the load of seed virus and phage (Bitton, 1980); these results agreed with data recorded on a water reclamation plant (Grabow, 1990).

For groundwater, available evidence indicates reasonable similarities in the behaviour of various enteric viruses and phages such as MS-2 (Yates, Gerber & Kelley, 1985; Sobsey, Hall & Hazard, 1995), and there is no reason to believe that the behaviour of enteric hepatitis viruses is significantly different. It therefore seems that existing recommendations for the utilization and treatment of groundwater (World Health Organization, 1996) can be trusted to cover hepatitis viruses.
Disinfection processes have been investigated in more detail. Laboratory experiments using a variety of viruses and phages, and detailed analysis of disinfection processes in water-treatment plants confirm that viruses and phages are at least 99% inactivated by recommended disinfection methods (Bitton, 1980; Grabow, 1990). Recommended conditions of disinfection include those made by the World Health Organization (1993, 1996) for conventional water treatment purposes, which specify a free chlorine residual of at least 0.5 mg/litre for 30 minutes at pH < 8.0, with mean turbidity not exceeding 1 nephelometric turbidity unit (NTU). Free chlorine residuals of 1 mg/litre and an exposure time of 60 minutes have been recommended for the disinfection of drinking-water directly reclaimed from wastewater (Grabow, 1990). In laboratory experiments, cell-culture-adapted strains of HAV were unable to survive these conditions of disinfection; the rate of inactivation resembled that of vaccine polioviruses and F-RNA phages (Grabow et al., 1983; Sobsey, 1989). Similarly efficient inactivation of cell-culture-adapted strains of HAV has been reported for other oxidizing agents such as ozone and ozone–hydrogen peroxide (Hall & Sobsey, 1993), and for other commonly used disinfection processes such as ultraviolet light irradiation (Wiedenmann et al., 1993).

Generally accepted goals for the overall efficiency of water-treatment plants include those of the United States Environmental Protection Agency, which specify a minimum 4 log (4 orders of magnitude) reduction in virus numbers in water sources of acceptable quality (Rose & Gerba, 1991). Evidence has been presented that conventional water-treatment plants operated in accordance with design specifications are capable of this level of efficiency (Bitton, 1980; Grabow, 1990; Ford & Colwell, 1996; World Health Organization, 1996). However, design specifications are usually based on the optimal functioning of all barriers in the system, which implies that any breakdown, malfunction, or suboptimal operation will impair the efficiency of the system. Highly resistant pathogens like viruses are the most likely to survive in such a situation. If loss of efficiency coincides with exceptionally high numbers of viruses in the raw water intake (because of seasonal fluctuations or a disease outbreak), there is a risk of infection (Hallerday et al., 1991). Events of this kind probably explain why viral hepatitis and certain other diseases are often associated with drinking-water supplies from plants apparently operating in accordance with accepted specifications (Bosch et al., 1991b; Ford & Colwell, 1996; Grabow, 1996, 1997).

The early detection of failures in water-treatment plants depends heavily upon the regular monitoring of quality. Unfortunately, considerations of cost often mean that monitoring is restricted to inexpensive, simple, and rapid tests, such those for coliform bacteria. These faecal bacteria are not reliable indicators of the presence of viruses, as evidenced by the frequency with which hepatitis and other viral infections are associated with drinking-water supplies that meet generally accepted criteria for faecal coliforms (Hejkal et al., 1982; Bosch et al., 1991b; Ford & Colwell, 1996; Grabow, 1996; Payment, 1997).

Despite exceptional resistance, there is no evidence that hepatitis or any other viruses can survive recommended conditions of water treatment and disinfection.
(World Health Organization, 1993, 1996). However, the production of safe drinking-water supplies requires rigorous and failsafe application of these specifications. Important aspects of the recommendations include the utilization of treatment processes appropriate according to source water quality, subject to periodic verification and continuous monitoring of process efficiency (World Health Organization, 1993, 1997). This will normally require frequent testing of physico-chemical indicators of treatment and disinfection processes. Shortcomings of conventional coliform indicators for monitoring the microbiological quality of water and the efficiency of treatment processes could be supplemented by using appropriately selected combinations of additional indicators, which could include highly resistant organisms such as *Clostridium perfringens*, phages, and heterotrophic plate counts (Ford & Colwell, 1996; Grabow, 1996). This would apply in particular to potentially high-risk situations such as the production of drinking-water from heavily polluted source water (Grabow, 1990). Ideally, of course, monitoring of treatment plants and drinking-water supplies should include tests for viruses, but this is not yet within the technical or financial reach of many water suppliers.

Recently introduced vaccines may offer protection against waterborne HAV. However, no vaccines are available for HEV and HFV, and there is no indication of any becoming available in the foreseeable future. Similarly, immunoglobulin preparations are available for temporary protection against HAV, but no such preparations are available for protection against HEV and HFV. There is no chemotherapeutic treatment for infections caused by any of these viruses—only supportive treatment. The benefits of HAV immunization are not altogether clear. Prevention of infection in endemic areas would require immunization of very young children. However, the vaccines consist of inactivated HAV and, unlike natural infection, do not confer lifelong immunity. Early immunization may simply shift susceptibility from childhood to later in life, when the impact of infection is more severe. This, like the similar situation that arose with poliovirus (Iwarson, 1992), is clearly undesirable. Moreover, the benefit for the growing number of immunocompromised individuals remains uncertain. For individuals in specific situations, such as health care workers in critical service areas, sewage workers, and military personnel deployed on short notice to high-risk areas, HAV immunization may indeed offer significant advantages. However, immunization does not seem to offer a practical or universal alternative to control based on prevention, i.e. safe water and food, and good standards of hygiene and sanitation.

**Conclusions and recommendations**

**Health risk assessment**

The enteric hepatitis viruses continue to be responsible for a significant disease burden—both through outbreaks of disease and through endemicity. They are all transmitted by the faecal–oral route, typically through drinking-water and
food in which they appear to be highly infectious. The only known reservoir of HAV and HFV is the human population. By contrast, recent evidence indicates that HEV is much more zoonotic than was previously thought, and a variety of domestic and wild animals may serve as reservoirs for the virus. This implies that faecal pollution of both human and animal origin may pose a risk of HEV infection.

The HAV vaccines that have been developed may be used to protect individuals in high-risk situations, but the principal barriers to transmission of enteric hepatitis virus are safe drinking-water supplies and good standards of sanitation and of personal and food hygiene. The relative importance of environmental transmission of enteric hepatitis viruses is likely to increase as demands on limited water resources encourage water reuse, as improvements in basic water treatment tend to eliminate the more susceptible pathogens (such as *Salmonella* and *Vibrio*), and as the age of initial exposure to the viruses increases.

Risk management

Risk management includes specifications for the quality of raw water sources and the efficiency of treatment and disinfection processes outlined earlier. Use of appropriate combinations of indicators for quality monitoring and assessment, and programmed sanitary surveys for pollution of human and animal origin should also be integral components of risk-management strategy.

Enteric hepatitis viruses are highly infectious. Although individual risk may be minimal in areas of low endemicity, there remains the risk of sporadic introduction of the viruses by symptomatic or asymptomatic individuals, especially returning travellers and their immediate contacts. Effective control where the water supply is derived from faecally contaminated sources requires multiple treatment barriers, including chemical disinfection. In areas of high endemicity and/or low water pressure or high leakage rates in distribution systems, the use of a residual disinfectant is required, with safe concentrations maintained throughout the distribution system.

Specific analysis of water for hepatitis viruses would be carried out only in exceptional circumstances—predominantly for research intended to elucidate how the viruses could have survived treatment and disinfection processes. Comprehensive quality surveillance programmes, designed to detect the earliest possible indications of quality fluctuations in raw water sources, treatment failure, doubtful final quality of the water, or relevant incidences of disease in communities concerned, are valuable tools for risk management (Grabow, 1996).

Research priorities

In view of the shortcomings of indicator systems and indirect quality assessment methods, development of practical methods for direct detection and monitoring of viruses remains a high priority (Sobsey et al., 1993; Ford & Colwell, 1996).
This is especially important for research on the behaviour of viruses in treatment and disinfection processes. The many questions about the epidemiology of enteric hepatitis viruses, such as the apparent geographical distribution of HEV and HFV, should also be addressed, since they are crucial to improved risk management and to the control of local waterborne transmission and worldwide dissemination of these viruses. Technological progress, particularly the development of molecular techniques, suggests that important new tools and information may soon become available.

References


