Hepatitis viruses in water: Update on risk and control

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Abstract

Three different hepatitis viruses, designated hepatitis A (HAV), hepatitis E (HEV) and hepatitis F (HFV) are now known to be transmitted by water. HAV has a long history of water-borne transmission in all parts of the world. HEV has been discovered only recently, and is now known to cause outbreaks of clinical disease in certain parts of the world. Indications are that HFV causes sporadic cases in restricted areas. Although the mortality of infections caused by all three viruses is relatively low, clinical disease may be severe and incapacitating. Case fatality rates of 20 to 40% are on record for HEV infections in pregnant women. HAV is endemic in most of the population of South Africa. Recent evidence indicates that HEV is also endemic, with high incidence in some communities. Although HFV has not yet been recorded in the country, it could be imported rapidly. The risk of water-borne hepatitis in South Africa should, therefore, not be underestimated. The risk can be expected to increase as a result of population growth and escalating demands on limited water resources. Since vaccines are available only for HAV, and no meaningful treatment is available for any of the viruses, control of the diseases depends on prevention of transmission. This implies a major responsibility for the water industry and related health authorities. No practical methods are available for direct detection of any of the viruses. Monitoring of the safety of water supplies does, therefore, continue to rely on the meticulous application of indirect methods. Shortcomings of these indirect methods emphasise the need for practical techniques to detect the viruses.

Introduction

The term "hepatitis viruses" refers to a diverse group of viruses which all have the human liver as primary target of replication. "Hepatitis" is derived from "Hepar" (Greek for "liver") and the suffix "-itis" which denotes "inflammation". According to the Babylonian Talmud, hepatitis was common in the 5th century BC, and Hippocrates described the disease in detail (Grabow, 1976; Zuckerman, 1983; Zuckerman and Thomas, 1993). The replication of hepatitis viruses 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 released from red blood cells during the ongoing replacement of these cells by new ones. The colour of bilirubin is yellow to green, and accumulation in the blood circulation system results in excretion through the kidneys (dark urine), the digestive tract (dark stool) and visibility as yellow colour in the peripheral blood network at sites such as the eyes and hand palms. This symptomatic condition of accumulated bilirubin is known as "jaundice". Another typical consequence of the massive liver cell damage is release of liver enzymes into the blood stream. These enzymes include alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the serum levels of which are used to diagnose hepatitis (Zuckerman and Thomas, 1993).

Hepatitis may also be caused by other systemic pathogens which do not have the liver as primary or only target of replication such as cytomegalovirus, yellow fever virus and *Leptospira* bacteria. Liver cell damage and jaundice may also be caused by toxins such as excess alcohol.

With regard to water quality analysis, hepatitis viruses share the important feature of not readily causing a cytopathogenic effect (CPE) in presently available cell culture systems, which implies that they are not detectable by conventional cell culture propagation procedures used for viruses like reo, polio and coxsackie. The hepatitis D virus is even a defective virus, which can only replicate with the assistance of the hepatitis B virus (Taylor, 1996). Also, hepatitis viruses are highly host-specific, which implies that few if any conventional laboratory animals can to a meaningful extent be used for research on most hepatitis viruses.

Since the clinical symptoms caused by hepatitis viruses may appear similar, and some of the viruses only emerged on a large scale in recent years, the first distinction of different aetiological agents was accomplished only in the 1960s. The first two hepatitis viruses to be distinguished, were simply 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. At this stage 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 uninformed. The nomenclature of the viruses is abbreviated as HAV to HGV for hepatitis A to G viruses.

Hepatitis viruses are divided into two basically different groups, some distinctive features of which are summarised in Table 1. One group is referred to as enteric hepatitis viruses, consisting of HAV, HEV and HFV. These viruses are primarily transmitted by the faecal-oral route, i.e., by water and food, and will in this paper be referred to as "water-borne hepatitis viruses". The second group consists of parenterally transmitted or blood-borne hepatitis viruses, including HBV, HCV, HDV and HGV. These viruses are primarily transmitted by blood and blood products, as in medical transfusion, as well as sexual intercourse, contaminated medical instruments like syringes and needles, and even by tattooing and insect bites. There is no evidence that blood-borne hepatitis viruses are of meaningful concern to water quality. HBV would seem to be inactivated by enzymes produced by bacteria in the gastro-intestinal tract and environmental waters

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TABLE 1 CLASSIFICATION OF HEPATITIS VIRUSES	
Hepatitis virus	**Characteristic features**
Water-borne	
Hepatitis A (HAV)	Family Picornaviridae; non-enveloped 27 to 32 nm spherical particles; ss-RNA, no chronic infection or carrier state; vaccines available; world-wide; low mortality
Hepatitis E (HEV)	Family Caliciviridae; non-enveloped 25 to 35 nm spherical particles; ss-RNA, no chronic infection or carrier state; no vaccines available; restricted geography; high mortality in pregnant women
Hepatitis F (HFV)	Unclassified; non-enveloped 27 to 37 nm spherical particles; ds-DNA; no chronic infection or carrier state; no vaccines available; apparently restricted geography and low mortality
Blood-borne	
Hepatitis B (HBV)	Family Hepadnaviridae; enveloped 42 nm spherical particles; ds-DNA; sometimes chronic carrier state; vaccines available; world-wide; sometimes complication of fatal liver cancer
Hepatitis C (HCV)	Family Flaviviridae; enveloped 30 to 38 nm particles; ss-RNA; chronic infection in >50% of cases; no vaccines available world-wide
Hepatitis D (HDV)	Classified as sub-viral agent; enveloped 35 to 37 nm spherical particles; ss-RNA; often chronic infection; no specific vaccines; restricted geography; defective virus dependent on HBV
Hepatitis G (HGV)	Family Flaviviridae; enveloped 35 to 37 nm spherical particles; ss-RNA; often chronic infection; no vaccines available; world-wide
ss = single-strand; ds = double-strand	

(Grabow et al., 1975). Infectious HBV is, therefore, rarely if ever detectable in faeces, water or food. The same would seem to apply to the other parenterally transmitted viruses HCV, HDV and HGV. Blood-borne hepatitis viruses will, therefore, not be discussed in further detail.

Hepatitis A virus

HAV has the longest and best known history of the three enteric hepatitis viruses presently known. Initially HAV was known as the "infectious" or "epidemic" hepatitis virus, because of its typical association with epidemics caused by contaminated water and food world-wide (Grabow, 1976). Extreme examples include an outbreak of some 300 000 cases in 1988 in Shanghai, China, caused by the consumption of clams harvested from a bay polluted with sewage from a community in which an epidemic of hepatitis A had occurred (Halliday et al., 1991). HAV shares many features with members of the genus Enteroviruses such as polio and coxsackie viruses, and has in the past been classified as Enterovirus type 72. More recently HAV has been classified in its own genus, first referred to as Heparnavirus, and now Hepatovirus. HAV infects epithelial cells of the gastro-intestinal tract, and may from there proceed to infect the liver. Enteroviruses like polio and coxsackie follow the same route of primary infection, but may in a small percentage of cases proceed to infect the central nervous system. It must be kept in mind that the gastro-intestinal tract starts at the oropharynx, and during early stages of infection these viruses are detectable in throat swabs and sputum specimens, and the viruses may also be transmitted by the oral-oral route.

Typical of Enteroviruses, HAV infection tends to be mild or without clinical symptoms in young children. The incidence of infection is closely associated with hygiene and sanitation, and in developing countries most individuals contract infections during early childhood. Fortunately there would seem to be only one antigenic type, which elicits lifelong immunity. In adult populations of developing countries and communities immunity to HAV may exceed 95%, in contrast to less than 50% in developed countries and communities (Iwarson, 1992; Sathar et al., 1994; Tucker et al., 1996). People from developed countries visiting the developing world are, therefore, exposed to risk of infection. HAV typically occurs in all parts of the world, and

there is no indication of geographical preferences other than those related to levels of hygiene and sanitation. Typical clinical symptoms of infection are predominantly seen in adults. Although the general level of mortality is less than 1%, the disease may be quite severe and incapacitating. The infection may cause substantial liver damage, and regeneration of the liver cells takes time (Zuckerman and Thomas, 1993). Implications are that patients may be confined to bed for up to six weeks or more, feeling ill with apathy towards foods which heavily depend upon liver functions for digestion. The severity of the disease and mortality may be associated with underlying conditions such as immunodeficiencies, malnutrition and general state of health.

Since HAV is not detectable by conventional routine cell culture procedures, many questions about the epidemiology of the virus as well as its incidence and behaviour in the environment remain unanswered. There is, however, little doubt that the virus is highly infectious, relatively resistant to unfavourable environmental conditions including water treatment and disinfection processes, and can cause explosive outbreaks when present in water or food. At least by implication, there is reason to believe that the minimal infectious dose is extremely low, possibly as low as a single infectious particle.

The history of research on HAV reads like a science fiction novel. Much of the available information on HAV was derived from experiments on human volunteers. Well-known studies include those of Neefe and co-workers (see Grabow, 1976) carried out during the second world war in attempts to control the disease which is notorious for having a devastating impact on troops as well as civilians under conditions prevailing during times of war. This was followed by experiments using mentally retarded children at the Willowbrook School in New York, which among other things resulted in the first distinction between HAV and HBV. It seems most unlikely that experiments of this kind would today be ethically approved. Shortly afterwards the virus was visualised by electron microscopy. The next breakthrough was the discovery of subclinical infection in some primates other than man (Grabow et al., 1981). This discovery was initiated by observations that chimpanzee handlers at US aeronautic and space research facilities displayed higher than normal incidences of HAV infection. It transpired that they contracted the infections from chimpanzees imported from hepatitis A endemic areas in Africa. These chimpanzees served as pioneer crew on the first experimental craft launched into space. Another milestone was the discovery that the virus can actually under circumstances replicate in certain cell cultures (Grabow et al., 1983), which led to the establishment of cell culture adapted strains of HAV (Gust et al., 1985). Although they may in some ways differ from wild-type HAV, these adapted strains 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 hybridisation and the polymerase chain reaction (PCR), made it possible to develop sensitive techniques for detection of the virus (Dubrou et al., 1991; Deng et al., 1994; Tsai et al., 1994).

Hepatitis E virus

The existence of HEV was discovered only in the late seventies and early eighties (Wong et al., 1980),after it had become evident that there was a hepatitis virus other than HAV and HBV. Initially, hepatitis E was referred to as enterically transmitted or epidemic non-A, non-B hepatitis. It transpired that for many years HEV had actually been mistaken for HAV, because they share certain basic clinical and epidemiological properties (Grabow et al., 1994; Purcell, 1996). Both viruses are transmitted primarily by the faecal-oral route, and are often associated with water- and food-borne outbreaks. However, 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 develops complications, hepatitis E tends to display more prominent cholestasis and can present as acute fulminating hepatitis, particularly in pregnant women, in which case fatality rates as high as 20 to 40% have been reported. Hepatitis E has an incubation period of about 40 d, which is slightly longer than that of hepatitis A, and has a lower secondary attack rate by personal contact. Viraemia would seem to be common for HEV infections, and may last for up to 16 weeks, which is longer than for HAV infections. Patients may excrete HEV for more than seven weeks, well after clinical and biochemical recovery (Clayson et al., 1995; Scharschmidt, 1995). Peak excretion of HAV is generally the week prior to the onset of clinical symptoms, after which the virus is rarely detectable in patient stool specimens.

Although HEV is a single-stranded RNA virus with non-enveloped 25 to 35 nm icosahedral capsid like HAV, it differs substantially at molecular level and has been classified as a member of the family Caliciviridae. Other well-known members of this family include the wide range of gastro enteritis viruses such as Norwalk, Marin County, etc. In contrast to HEV which may infect the liver, infection and replication of the closely related gastro-enteritis viruses would seem to be confined to epithelial cells of the gastro-intestinal tract. There are indications of antigenic variation and even differences in serotypes of HEV (Chauhan et al., 1994; Purcell, 1996), which may have implications for immune status and immunological assays used for diagnostic purposes (Mast et al., 1996). This would imply a substantial difference from HAV.

The epidemiology of HEV displays marked differences from that of HAV. Although both are primarily associated with low hygiene and poor sanitation, the epidemiology of HEV would seem to also include a geographic element (Grabow et al., 1996b). Clinical infections and outbreaks of hepatitis E have been recorded predominantly in certain parts of the world such as India, Nepal, Burma, Pakistan, Afghanistan, Borneo, parts of Central Asia and China, and then in Mexico, and also in parts of Africa such as Egypt, Algeria, Ethiopia, Somalia, Sudan, and the Ivory Coast. The disease is endemic in many of these countries, and is the most common cause of acute hepatitis in adults in parts of India, Asia and Africa. Large reported outbreaks associated with sewage-contaminated drinking water include one in 1954 involving approximately 40 000 cases in Delhi, India. For more than 20 years this outbreak was thought to have been caused by HAV (Purcell, 1996). When technology for HEV became available, stored sera of patients were re-investigated and antibodies revealed that the outbreak was actually caused by HEV. Furthermore, there were hepatitis E outbreaks with more than 100 000 cases in 1986 to 1988 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 even more so, would seem to occur rarely in parts of the world such as central Europe, Britain, North and South America, Australasia, Japan and South Africa (Craske, 1992; Grabow et al., 1996b). Clinical cases in these parts of the world tend to be limited to imported cases. However, seroprevalence studies now reveal that the virus is actually present in many of these countries, and 2 to 10% of inhabitants may have antibodies, which confirms exposure to the virus. Reasons for a relatively low incidence of clinical

cases and outbreaks in certain parts of the world despite the presence of the virus are not yet fully understood (Grabow et al., 1996b). Answers to these and related questions are of fundamental importance because they may hold the key to strategies for preventing world-wide spread of the virus and controlling the disease (Scharschmidt, 1995).

Since HEV is not readily detectable by conventional routine cell culture procedures, most of the initial studies on the virus were confined to experiments with human volunteers, electron microscopy, immunological assays, and epidemiological data. Important progress has been made in studies using exotic animals such as cynomolgus macaques, tamarins and chimpanzees in which the virus seems to cause an infection resembling that in humans (Bradley et al., 1987). Research progress really only accelerated when molecular techniques became available (Favorov et al., 1992; Jothikumar et al., 1993). It would now appear that HEV has zoonotic features and that there may be animal reservoirs for the virus (Clayson et al., 1996), which would imply another substantial difference from HAV. Evidence has been presented that at least some strains of HEV may replicate in more readily available animals such as laboratory rats (Maneerat et al., 1996), domestic swine (Balayan et al., 1990), rhesus monkeys (Nanda et al., 1994) and even some cell cultures (Huang et al., 1992;1995; Meng et al., 1996; Tam et al., 1996). If these initial findings prove to be of practical significance, they will substantially facilitate research on the virus.

Hepatitis F virus

The limited information available on HFV suggests that it is transmitted by water and food like HAV and HEV (Craske, 1992). In contrast, however, HFV is associated with sporadic cases in certain geographical areas, and not with outbreaks or epidemics (Sharma et al., 1990; Deka et al., 1994). Cases have been recorded in the UK, Italy, the USA and India, but not yet in South Africa. In some of these areas clinical cases of HEV are virtually unknown and HAV rare. Reasons for this epidemiology are not clear. The virus would seem to consist of a non-enveloped icosahedral particle with a diameter of 27 to 37 nm containing double-stranded DNA. Infection of rhesus monkeys has been reported, and so has replication with cytopathogenic effect in the Hep-2 (human larynx carcinoma) cell line. The virus has not yet been classified. 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 viral hepatitis. Typical of hepatitis viruses, HFV would not seem to be detectable by conventional routine cell culture techniques.

Risk of water-borne hepatitis

Very few if any details are available on the water-borne transmission of hepatitis viruses in South Africa. This is almost certainly due to the absence of an infrastructure and research endeavours aimed at the detection and recording of such incidences. Although viral hepatitis is a notifiable disease in South Africa, there is no doubt about gross under-reporting, and notifications give no indication of the source of infection (Bourne and Coetzee, 1996). Hepatitis A is known to be endemic in most of the population of South Africa (Grabow et al., 1994; Sathar et al., 1994). Infections are regularly recorded by diagnostic laboratories (unpublished data), and according to unconfirmed word of mouth, outbreaks occur regularly, but no meaningful effort has yet been made to investigate the epidemiology. In view of the high incidence of infections, the virus must be present in the environment at relatively high density. Since there is no reason to believe that the basic mode of transmission may differ from that in parts of the world where water-borne transmission has been described in detail, it would, therefore, seem logical that the risk of water-borne hepatitis in South Africa must be relatively high. In view of population growth, escalating demands on limited water resources, and demographic developments such as rapid urbanisation, the risk of water-borne hepatitis can be expected to increase because these and related developments create conditions in which HAV thrives.

Details on HEV in South Africa which are now beginning to surface, are reason for concern. Until recently HEV was not known to occur in the country. However, the latest seroprevalence data from a number of sources leave no doubt that the virus is actually endemic in the country, and that up to 19% of individuals in certain low socio-economic communities are infected (Grabow et al., 1994, 1996b; Tucker et al., 1996). Initially it was thought that clinical cases in the country were limited to a small number of imported cases, but it would now appear that there may be more local cases of clinical disease than previously thought (Aspinall et al., 1995; Grabow et al., 1996b). However, water-borne outbreaks like those on record for many parts of the world remain unknown, and the incidence of clinical cases would still appear relatively low, more closely resembling that in industrialised countries than in the developing world. This appears surprising since general conditions of hygiene and sanitation in low socio-economic communities in South Africa, notably informal settlements (Grabow et al., 1996a), would seem to closely resemble those where clinical hepatitis E and outbreaks of the disease are common. Concern about the disease in South Africa is supported by recent evidence of typical outbreaks with clinical cases in neighbouring countries Botswana and Namibia (Swanepoel et al., 1995; Grabow et al., 1996b).

Hepatitis F has not yet been reported in South Africa. However, there would not seem to be any reason to believe that the virus may not already be in the country, or that it could not surface at any time. Conditions in many communities are ideally suited for enteric pathogens such as HFV, and large-scale movement of people locally and across all international borders can rapidly result in the importation and spread of new pathogens (Grabow, 1996b), as has been illustrated by imported clinical cases of hepatitis E (Grabow et al., 1996b), and by an imported case of Ebola virus infection in October 1996.

Control of water-borne hepatitis

The elimination of hepatitis viruses from water, at least within acceptable limits, is possible and feasible. Like any other pathogens, these viruses can satisfactorily be removed and/or inactivated. However, as a result of cost, available expertise and facilities, and factors such as human negligence and error, this is not always accomplished. Important in this regard are factors such as the variable incidence of viruses in raw sources, and the exceptional resistance of at least HAV. The incidence of these viruses in raw sources depends on epidemiological features, i.e., an outbreak in a particular community may result in high levels of viruses in raw sources which substantially increases the risk of viruses surviving treatment and disinfection processes. This has clearly been illustrated by the 1988 outbreak of hepatitis A in Shanghai referred to earlier. The exceptional resistance of viruses implies that they would be among the first to pass in

meaningful numbers through inadequate treatment and disinfection processes, which is reflected by data on water-borne diseases world-wide (Regli et al., 1991).

HAV survival of inadequate water treatment and disinfection even when meeting generally accepted specifications for indicators and treatment efficiency, has been proven by epidemiological studies as well as water quality analysis and laboratory studies on the survival and behaviour of HAV compared to that of commonly used indicators. For instance, outbreaks of hepatitis associated with drinking water supplies which conformed to specifications, have been referred to earlier. In at least some of these studies the epidemiological conclusions were supported by detection of HAV in the water supplies concerned (Hejkal et al., 1982; Bosch et al., 1991b). Exceptional resistance of HAV to water treatment and disinfection processes has been confirmed in a number of laboratory experiments (Grabow et al., 1983; Rao et al., 1988; Mbithi et al., 1990; Hall and Sobsey, 1993; Nasser et al., 1995), and behaviour dissimilar to that of at least some indicators and other viruses in natural water environments has been described in many studies (Tsai et al., 1993; Callahan et al., 1995; Lévêque et al., 1995; Sobsey et al., 1995).

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

Recently introduced HAV vaccines may contribute to the control of this virus. There are no vaccines available for HEV and HFV, and there is no indication of vaccines for these viruses in the foreseeable future. The same applies to immunoglobulin preparations which could be used for temporary protection as practised in the case of HAV. There is no chemotherapeutic treatment for infections caused by any water-borne hepatitis viruses, other than supportive treatment. At least in the case of HEV and HFV control is, therefore, entirely based on prevention of transmission, i.e. the safety of water and food, and general hygiene and sanitation. Benefits of the HAV vaccines are not yet altogether clear (Iwarson, 1992; Martin, 1992). The HAV vaccines would have protective value for susceptible individuals from developed countries visiting high-risk developing areas where HAV is endemic. Others for whom vaccination may be beneficial would include health-care workers in critical service areas, staff of child-care centres, sewage workers and the military who could on short notice be deployed to high-risk areas. The benefit for populations in high-risk parts of the world where lifelong immunity is acquired free of charge by natural sub-clinical infection, remains uncertain, particularly because these people are not likely to have the financial resources for mass vaccination. Prevention of infection in endemic areas would require vaccination of children at a very young age. Since the vaccines consisting of inactivated HAV may fail to confer life-long immunity like natural infection (Iwarson, 1992), such vaccination may shift susceptibility from childhood to later in life when the impact of infection is more severe. This may create an undesirable situation similar to that when susceptibility to the polio virus was shifted from childhood to later in life.

Monitoring water supplies for hepatitis viruses

Meaningful technology and expertise for the detection of hepatitis viruses in water are limited to HAV. Detection of HEV in water has been reported only once (Jothikumar et al., 1993), and HFV never yet. Indications are that HAV can successfully be recovered from water using techniques commonly applied for other enteric viruses such as polio, coxsackie and reo. This would include adsorption-elution procedures using positively or negatively charged membrane filters followed by organic flocculation for secondary concentration (Sobsey et al., 1985). HAV has been recovered from seeded drinking water samples by means of ultrafiltration at an efficiency of recovery (EOR) of 100%, which was higher than that of polio viruses (Divizia et al., 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. EOR for HAV in seeded 20 litre samples was 100% for tap water, 94% for sea water and 61% for fresh water and sewage. HAV has also been recovered from sewage sludge (Graff et al., 1993), shellfish meat suspensions (Deng et al., 1994; Jaykus et al., 1996) and drinking water supplies (Schwab et al., 1996) by means of antigen capture techniques using HAV-specific antibodies to recover the virus, but no specific details on EOR are available. Using a glass wool adsorption-elution procedure (Grabow and Taylor, 1993), HAV has been recovered from seeded drinking water samples at an EOR in excess of 60% (Grabow et al., unpublished data).

Regarding the recovery of HEV, the only available details are those of Jothikumar et al. (1993) who successfully recovered the virus 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 Enteroviruses such as polio virus which is recovered at this pH level in some routine techniques.

The next step in water quality analysis and monitoring after recovery of viruses is their detection. Most of the above experiments on recovery of HAV were carried out using cell culture adapted strains of HAV which can be titrated by conventional cell culture procedures or plaque assays. However, detection of wild type HAV requires different strategies, and presently the most feasible approach is based on molecular techniques. HAV has successfully been detected in environmental specimens directly by gene-probe hybridisation (Jiang et al., 1986; Dubrou et al., 1991) or PCR (Goswami et al., 1993; Graff et al., 1993; Tsai et al., 1993; Jaykus et al., 1996; Schwab et al., 1996). Goswami et al. (1993) claimed levels of sensitivity of ten 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 polio virus than conventional cell culture detection, which suggest that the same procedure would also be extremely sensitive for HAV. Tsai et al. (1994) developed a triplex RT-PCR which at the same time detects polio virus, HAV and rotavirus, extensively reducing time, cost and labour for the monitoring of water supplies. Supplementation of molecular techniques by prior cell culture amplification of viral RNA would seem to substantially increase detection sensitivity with the additional benefit of knowing that viable HAV has been detected (Dubrou et al., 1991; Shieh et al., 1991).

TABLE 2 INDIRECT ASSESSMENT OF WATER FOR THE ABSENCE OF HEPATITIS VIRUSES
1. Appropriately selected combinations of indicators for assessment of faecal pollution and the survival of enteric viruses.
2. Routine sanitary survey of raw sources, treatment processes, and the distribution of treated water.
3. Specifications for the quality of raw sources and the efficiency of treatment and disinfection processes.
4. Comprehensive quality surveillance programmes on raw sources and treated supplies, the efficiency of treatment and disinfection processes, and the epidemiology of enteric diseases in the communities concerned.

HEV detection in water has been reported only by Jothikumar et al. (1993) who used a PCR procedure based on preparing HEV-specific cDNA by reverse transcription for amplification by PCR and detection by slot blot hybridisation. Information on the role of water 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 sero-prevalence studies, i.e., 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 denotes transmission by water and food (Grabow et al., 1996b).

The above is a basic summary of technology presently available for the detection of hepatitis viruses in water. Although excellent progress has been accomplished, established methods are at this stage limited to HAV. In view of basic differences outlined earlier, it is possible that the behaviour of HEV and HFV in water environments may differ from that of HAV. In addition, the technology for HAV is not yet within easy reach of the expertise and facilities of many water quality laboratories. Consequently assessment of the safety of water supplies continues to depend largely upon indirect methods, the most important of which are summarised in Table 2. Indicator organisms remain important. However, presently available information leaves no doubt that indicators commonly used have shortcomings with regard to hepatitis viruses as well as other enteric viruses (Grabow, 1996a;b). For instance, outbreaks of hepatitis A have been associated with water supplies which conformed to generally accepted guidelines for indicators and treatment procedures (Hejkal et al., 1982; Bosch et al., 1991b; Grabow, 1996a). Also, laboratory experiments have proved that HAV, as well as at least some other enteric viruses, are more resistant to unfavourable conditions, including water treatment and disinfection processes, than commonly used indicators such as coliform bacteria.

Indicators do, therefore have to be applied with caution. This implies that combinations of indicators appropriate for various purposes have to be used (Grabow, 1996a). These batteries of indicators may have to include indicators such as phages, *Clostridium perfringens*, and at least those enteric viruses that are detectable (Grabow, 1996a). The importance and value of sanitary surveys have been outlined previously (Grabow, 1996a; WHO, 1996), and so have strategies based on specifications for the quality of raw sources and the efficiency of treatment and disinfection processes (Lloyd and Bartram, 1991; Regli et al., 1991; Sobsey et al., 1993; States and Sykora, 1995; Grabow, 1996a). The value of comprehensive quality surveillance programmes including details on raw sources, the efficiency of treatment processes, the quality of water in distribution networks, the epidemiology of water-borne diseases in communities concerned, and related information, has proved of major value and this approach to monitoring the safety of water supplies is rapidly gaining ground (Grabow, 1996a;b).

Conclusions and recommendations

Despite modern technology and expertise the transmission of pathogens by water continues to occur (Grabow, 1996a;b). The challenge to control this route of transmission is likely to become more complex and difficult as demands on limited resources escalate and the need for reuse increases. These changes are reflected by trends in incidences which tend to shift from diseases caused by bacterial pathogens such as *Salmonella*, *Shigella* and *Vibrio*, to those caused by viruses and protozoan parasites which are more resistant to treatment processes (Grabow, 1996b). Controlling water-borne viral hepatitis is particularly important because no treatment for the diseases is available, and vaccines are available only for HAV. The only approach to controlling water-borne hepatitis is, therefore, based on prevention of transmission, which confers a major responsibility on the water industry and related health authorities because the diseases concerned have far-reaching public health implications.

The current situation with regard to water-borne viral hepatitis as outlined above, underlines the need for ongoing research on these viruses. This would include endeavours aimed at finding answers to many questions about the epidemiology of water-borne hepatitis viruses, such as the apparent geographic distribution of HEV and HFV. Answers to these questions are of fundamental importance in the formulation of meaningful strategies for controlling the water-borne transmission of these viruses locally, as well as their dissemination world-wide. Research should include the development of practical techniques for direct detection of the viruses. This technology is required for research on the incidence and behaviour of the viruses in water environments, which is essential to assess risks of infection, and determine the efficiency of water treatment and disinfection processes. Practical techniques for detection of water-borne hepatitis viruses are also required for monitoring the safety of water supplies in order to overcome the shortcomings of indirect methods of water quality monitoring presently used. Fortunately, current progress in

technology and expertise, particularly those based on molecular biology, has paved the way for attractive possibilities and sound progress.

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