GLOBAL WATER PATHOGEN PROJECT

PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND EPIDEMIOLOGY ASPECTS

HEPATITIS A

Wim van der Poel
Central Veterinary Institute - Wageningen UR
Wageningen, Netherlands

Autur Rzezutka
National Veterinary Research Institute, Pulawy
Puławy, Poland

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Summary

Hepatitis A virus (HAV) is a significant cause of infectious hepatitis and a major cause of enterically transmitted infectious hepatitis. Yearly there are about 1.5 million clinical cases of HAV infection but it is estimated that the actual rate of infection is probably as much as ten times higher. Endemic levels of HAV are related to hygienic and sanitary conditions with high-income regions having very low endemic HAV levels and low-income countries having high levels of endemicity. HAV is a nonenveloped icosahedral virion about 27 nanometers in diameter. Virions consist of single-stranded, plus sense RNA about 7,500 nucleotides long. There is a poly(A) tract on the 3' end, within a protein capsid shell consisting of four structural proteins, designated VP1-4. Various primary and continuous primate cells will support culture adapted HAV growth. Cell lines derived from African green monkey kidney (AGMK) cells such as (BS-C-1, Vero, and BGMK), fetal rhesus kidney (FRhK4) or human hepatoma (PCL/PRF/5) tissue have been useful for studying replication and for propagating high viral titers. Many wild-type strains, however, have not been adapted to cell culture despite intensive efforts, and successful isolation from clinical specimens often requires several weeks of blind passaging. Natural infection usually occurs 3 to 4 weeks following ingestion of virus from material contaminated with human feces containing HAV. The progression of events from ingestion of HAV to replication in the hepatocyte has not been completely resolved. During incubation, viremia occurs concurrently with fecal shedding of HAV due to release of viruses via bile. There is no specific treatment for acute viral hepatitis with therapy designed to be supportive and maintaining adequate nutritional balance. Although the disease is usually mild in children under 10, those who become clinically ill are more prone to develop fulminant hepatic failure compared with those who develop clinical HAV infection between the ages of 10 to 14 years. At older than 40 years, the severity of the liver disease intensifies. HAV is on of the most common causes of infectious jaundice in the world today with routine recurrent epidemics.

An HAV isolate that can be distinguished from other HAV isolates is defined as a strain. Viruses recovered from different locations often represent different strains, whereas viruses from individuals in the same outbreak usually represent the same strain. Well-characterized strains include HM175, CR326, MS-1, SD 11, GBM, and H2, all collected from humans. Analysis of a 168-base region of 152 HAV strains resulted in the designation of seven genotypes (<85% sequence identity between genotypes and no more than 15% divergence within a genotype). The vast majority of human strains were genotype 1 with genotype III representing most of the remaining human strains.

The level of sanitation and hygiene in the population markedly influences the epidemiology of hepatitis A. In crowded, unhygienic conditions, prevalence studies reported that infections occur at an early age and that close to 100% of children acquire immunity during the first decade of life. The principal mode of transmission is fecal-oral, resulting in community-wide outbreaks. High concentrations of virus are shed in the feces 3 to 10 days before the onset of clinical symptoms with infectious virions present in stool as early as 2 to 3 weeks before, and up to 8 days after the onset of jaundice.

Safe and effective inactivated vaccines have been developed for HAV with SmithKline Beecham producing HAvrix vaccine based on the HM175 strain adapted to MRC5 cells and Merck producing VAQTA vaccine based on the CR326F strain adapted to MRC5 cells. Both of these vaccines have proven to be safe and immunogenic in humans with only mild, clinically insignificant reactions.

Unpurified, cell-adapted HAV retains most of its infectivity when subjected to pH 1.0 for 2 hours at room temperature, and is still infectious at 5 hours, whereas poliovirus, coxsackievirus and echovirus lose nearly all infectivity by two hours. As a nonenveloped virus, HAV is also resistant to lipid solvents such as chloroform or vertrel and is resistant to heat. When incubated in neutral pH solutions at 60°C, HAV had no loss of infectivity and was only partially inactivated after 10 hours. HAV infectivity was preserved for more than one month following drying and storage at 25°C and for years following storage at -20°C or lower. HAV can persist for days to months in experimentally contaminated water, wastewater, soils or oysters. HAV can be inactivated by chlorine (10 to 15 ppm residual chlorine concentration after 30 min or free residual chlorine of 2.0 mg/L for 15 minutes).

Removal of HAV by conventional physicochemical water treatment processes of coagulation-flocculation and filtration is similar to that of other enteric viruses, with reductions up to 2 $\log_{10}$. Disinfection of water with free chlorine, chlorine dioxide, ozone, and ultraviolet light radiation can achieve up to 4 $\log_{10}$ inactivation of HAV under optimum conditions. Disinfectant contact time (CxT) four log values are, 20 mg-min/L for free chlorine, < 1 mg-min/L for ozone, and < 40 mg-min/L for chlorine dioxide. If HAV is protected by organic material or particles, however, rates of inactivation can be dramatically reduced and CxT values can be more that tenfold higher than for unblocked virions.

Hepatitis A

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

Approximately 1.5 million clinical cases of hepatitis A occur worldwide annually. However, this figure seems to be underestimated and the probable rate of infection is much higher (Franco et al., 2012). The incidence of hepatitis A varies from country to country, being related to sanitary conditions (Franco et al., 2012), demographic factors (Bura et al., 2012) and the socioeconomic status of the inhabitants (Franco et al., 2012). Differences in the

geographical occurrence of hepatitis A are also observed between world regions (Vaughan et al., 2014). Most infections occur in Africa, Asia, and Central and South America, followed by Central and Eastern Europe, which are considered areas of moderate endemicity. The yearly incidence rate of hepatitis A in Europe is between 0.55 and 1.5 cases per 100,000 citizens (Blachier et al., 2013). In developing countries hepatitis A virus (HAV) infections are very common, even reaching 100% of the population in the first years of life (Franco et al., 2012). Due to better sanitary conditions less evidence of hepatitis A is found in North America, Western and Northern Europe and Australia than in other regions (Wasley et al., 2006). These low endemicity regions are not free from large community food- or waterborne outbreaks and usually it is in the adult risk groups where disease is noted.

1.1.1 Global distribution

Several HAV (sub)genotypes can infect humans, with subgenotype IA being most prevalent worldwide and more frequently detected than IB (Desbois et al., 2010). Co-circulation of subgenotypes IA and IB has been reported in Europe and North America, with a higher prevalence of IA subgenotype virus strains in Europe. Other viruses belonging to genotypes II and III have also been reported in Europe (Nainan et al., 2006; D'Andrea et al., 2015). However, in some Asian countries an increasing prevalence of subgenotype IIIA strains has been observed (Nainan et al., 2006; Yun et al., 2011; Lee et al., 2012; Ishii et al., 2012). Although subgenotype IIIA seems to be endemic, mostly circulating in Asia, its emergence in Europe has caused outbreaks in Sweden, the UK, Estonia and Norway (Stene-Johansen et al., 2005).

1.1.2 Symptomology

Hepatitis A still remains a global public health concern. Disease occurrence is correlated with the socioeconomic status of inhabitants and access to clean water and sanitation (Rzezutka and Cook, 2014). Therefore in less developed countries the number of cases is usually high, which facilitates constant virus transmission. Currently, about 100,000 cases of HAV and about 500 deaths are reported annually in the WHO European Region (FitzSimons et al., 2010).

1.2 Taxonomic Classification of the Agent(s)

HAV is classified within the *Picornaviridae* family. Present classification of HAV strains is based on differences and homology of variable regions within the capsid protein. Particularly significant heterogeneity between virus isolates occurs within the VP1-2A junction (Robertson et al., 1992), the VP1 viral capsid protein (Costa-Mattioli et al., 2002) and the VP1-P2B region (Nainan et al., 2006). HAV can thereby be classified into six genotypes. Genotypes I, II and III have been isolated from humans, whereas genotypes IV, V and VI are of simian origin. Virus genotypes of human origin are further divided into subgenotypes A and B (Costa-Mattioli et al., 2002; Endo et al., 2007). Virus genotypes IV and VI were isolated from the cynomolgus monkey (*Macaca fasicularis*) and the African green monkey (*Cercopithecus aethiops*) respectively (Nainan et al., 2006).

1.2.1 Structure of the virion

The virus particle has an icosahedral capsid of about 27 to 32 nm with no envelope and contains a single-stranded RNA genome of approximately 7.5 kb. Each virus capsid is composed of 32 capsomers or protein subunits. The virus genome consists of several segments with one open reading frame translated into a single precursor polyprotein which is post-translationally cleaved to generate the functional viral proteins (Nainan et al., 2006). At the 5' end viral RNA is linked to the VPg protein followed by a 5' noncoding region (NCR) and the P1–P3 regions (Kumar et al., 2010). A short poly(A) tail which acts as the terminator of translation is located at the 3' end (Hollinger and Emerson, 2011). The P1 genome segment encodes capsid polypeptides (VP1, VP2, VP3 and VP4), whereas the P2 and P3 regions encode seven non-structural proteins 2A–2C and 3A–3D respectively, which are necessary for RNA synthesis and virion formation. The VP1 and VP3 capsid proteins act as an antigenic epitope on the viral surface which elicits a neutralizing antibody response (Nainan et al., 2006). The 5'-NCR region is the most conserved among all HAV strains isolated to date and it is frequently used to enable molecular detection (Houde et al., 2007).

1.2.2 New variants

As with other picornaviruses, recombination between HAV strains is possible, especially when co-infection of a single host cell with different virus strains takes place. In theory, recombination events could occur among the same or different virus genotypes. For example, recombination events between subtypes IA and IB have previously been reported but there is no evidence for recombination between subtypes IIIA and IIIB or other virus genotypes (Belalov et al., 2011). Despite minor sequence changes having appeared through virus evolution, they did not result in the formation of new serological variants, as all currently known HAV strains show a high degree of antigenic and genetic conservation even on the genotype level (Costa-Mattioli et al., 2003; Nainan et al., 2006). The HAV mutaton rate is the lowest among the members of the *Picornaviride* family, which may be responsible for its antigenic stability (Cristina and Costa-Mattioli, 2007).

1.3 Transmission

1.3.1 Routes of transmission

HAV transmission occurs via the fecal-oral route by direct contact with a sick person or asymptomatic virus shedder, or in rare cases by a sexual or parenteral route. Exposure to contaminated water and fomites or the consumption of contaminated uncooked food such as shellfish, fruit or vegetables could result in infection. For example, virus-contaminated frozen strawberries led to a multistate outbreak of hepatitis A among children and school employees in the USA. An epidemiological investigation revealed that contamination most likely took

place during harvesting of the berries by infected field workers (Hutin et al., 1999). Mixed frozen berries were also identified as the vehicle of infection in a multinational outbreak of the disease recently recorded in Europe (Severi et al., 2015, Terio et al., 2015). A common vehicle of virus transmission is sewage-contaminated water used for shellfish farming or irrigation of food crops. As demonstrated in several HAV outbreaks related to shellfish consumption, the sources of mollusk contamination were illegal waste disposal from recreational boats within legal harvest areas or illegal harvesting in closed areas (Bialek et al., 2007; Shieh et al., 2007). Compared to foodborne virus transmission, HAV transmission via drinking water or exposure to fecally-contaminated recreational water is less common. Nevertheless, several outbreaks traced to waterborne viruses have been reported (Rzezutka and Cook, 2014).

Hands are important vehicles for the transmission of enteric viruses, including HAV (Kotwal and Cannon, 2014). It was shown that extensive food handling during production, processing or packaging could result in fresh produce contamination and lack of observance of good personal hand hygiene allowed viruses to enter the food chain. For instance, in an outbreak of hepatitis A linked to the consumption of blueberries, an epidemiological investigation indicated that fruit contamination by HAV occurred on the farm. Poor sanitary facilities on the field did not facilitate proper hand hygiene among the berry pickers (Calder et al., 2003). Another example could be a Belgian outbreak, in which 269 individuals were infected with HAV. In this case an infected food handler working with bare hands at the meat processing plant caused the contamination (Robesyn et al., 2009). In addition, food preparation surfaces and kitchen utensils contaminated by viruses can also serve as an environmental route of virus transmission when produce is in contact with them. It has been shown under experimental conditions that in the action of cutting, HAV transfer can easily occur from contaminated produce to utensils and onward transfer can occur from contaminated utensils to uncontaminated produce (Wang et al., 2013). Viruses transferred to the surfaces of fruit and vegetables can persist sufficiently long between a contamination event and consumption to constitute a significant risk to consumer health (Rzezutka and Cook, 2014).

1.3.2 Reservoirs

In developing countries most infections are acquired during early childhood and in more than 50% of cases they have an asymptomatic course (Wu and Guo, 2013). In these settings, infected children play a major role in virus transmission to susceptible individuals and sustain the spread of the virus within the community. Several HAV genotypes have also been detected in non-human primates. Although there is no scientific evidence for cross-species transmission of HAV strains of primate origin to humans, the risk of exposure to these virus strains via food and water exists. Recently, the detection in irrigation water of a novel, possibly simian HAV strain genetically related to genotype V viruses was described (Rachida et al., 2016). Although simian strains can replicate in humans they do not produce disease (WHO, 2000).

1.3.3 Incubation period

The mean incubation period of the disease is around 30 days, with a maximum time of 50 days (Koff, 1992). During active virus replication, infected individuals generally remain asymptomatic. A viremia precedes the development of symptoms and virus is present in the blood of an infected person several weeks before symptom onset (Bower et al., 2000).

1.3.4 Period of communicability

1.3.4.1 Shedding levels

HAV is detected in stool just before IgM is detectable in serum (Matheny and Kingery, 2012). Feces may contain $>10^6$ infectious virus particles per gram (Greening, 2006). Virus excretion in feces can occur two to three weeks before symptoms appear (Hollinger and Emerson, 2011) and last for at least six weeks after their onset (Pintó et al., 2010). The concentration of the virus in stool declines after jaundice appears (Tassopoulos et al., 1986). In children and the immunocompromised virus shedding in feces may last four or five months after infection (WHO 2000). Chronic virus shedding has not been reported (Wasley et al., 2006). Besides in feces, HAV can also be found in the urine and saliva of those infected (Mackiewicz et al., 2004; Nainan et al., 2006; Mahboobi et al., 2012). The viral load in serum is 2 to 3 $\log_{10}$ units lower than that found in stool (Nainan et al., 2006).

1.3.4.2 Pathogenesis of the disease

The viral dose sufficient to cause infection is unknown, but is very low and likely to be between 10–100 $TCID_{50}$ (Boone and Gerba, 2007). After ingestion HAV replicates in the alimentary tract, from where the virus passes to liver tissue. In the liver viruses bind to HAVcr-1 receptors present on the hepatocytes, then enter the liver cells where they replicate efficiently (Kumar et al., 2010). The process of cell infection comprises the virus entry step, in which intact virus particles may pass through the cell membrane, followed by the viral RNA release step (Wang et al., 2015). From the liver mature viruses are released as quasi-enveloped virions which lose their host membrane envelope in biliary canaliculi. Subsequently viruses are transported via bile to the intestines and excreted in feces as naked particles (Walker et al., 2015).

The severity of disease is age-dependent with milder symptoms reported in children than in adults. In children under six years of age asymptomatic infections are common, although severe diarrhea in some children has also been reported. Acute liver failure can result in death in individuals over 40. Besides the general symptoms, there are also specific symptoms observed in the gastrointestinal and respiratory tracts including skin symptoms. In the icteric phase neurological symptoms may appear. In some cases, atypical manifestation of disease such as acute kidney injury has been reported (Jeong and Lee, 2010). Infection during pregnancy is associated with maternal

complications and preterm labor. There is no evidence of chronic liver disease or persistent infection. Complete clinical recovery takes place within two months in about 60% of patients, and within six months almost 100% become healthy with no subsequent liver disease.

Three classes of antibodies appear as the humoral immune response to HAV. Immunoglobulin M (IgM) antibodies are detectable before symptoms of infection appear. Within a few months they are gradually replaced by IgG antibodies which confer lifelong immunity. Although IgA antibodies are detected in serum, saliva and body excretions their role in host defense is not currently fully elucidated (Nainan et al., 2006).

1.3.5 Population susceptibility

In developed countries the changing pattern of HAV epidemiology is shifting from children to adolescents and young adults who are becoming increasingly susceptible to HAV infections (Franco et al., 2012; Campagna et al., 2012). The significant increase in anti-HAV seropositivity in sporadically vaccinated adults from countries with low HAV endemicity levels was linked to travel-associated infections and to the increased occurrence of behaviorally related risk factors (Wu and Guo, 2013).

1.4 Population and Individual Control Measures

Vaccination seems to be an important preventive measure in reduction of the risk of HAV infection. Besides prophylactic application of the vaccines they can be also administered as post-exposure prophylaxis against HAV (MMWR, 1996). Based on the socio-economic development and sanitation, current epidemiological situation or risk assessment data, national authorities could advise implementation of a vaccination program (Hendrickx et al., 2008). In areas of low risk of infection the recommended population groups for hepatitis A vaccine are those at higher risk of infection, for example injecting drug users, homosexual men and international travelers visiting regions of high HAV endemicity (Jeong and Lee, 2010). In addition, informing travelers of the risk of HAV infection when traveling to high risk regions and the possibility of vaccination may be relevant to prevent travel-related infections (Wu and Guo, 2013). In endemic countries infections are usually acquired early in childhood, which effectively prevents clinical hepatitis A in adolescents and adults. The level of immunity in the population is high, which recommends against mass vaccination (WHO, 2012). Although mass vaccination is not recommended in areas of high infection occurrence, it is suggested in regions of intermediate endemicity with periodic outbreaks of the disease (Nothdurft, 2008) or in countries receiving high numbers of immigrants from endemic regions (Pintó et al., 2010). It could also be used as a public health measure to control community-wide outbreaks (Craig et al., 1998). In some circumstances passive immunization can be recommended as post-exposure or pre-exposure prophylaxis of children or individuals at increased risk of infection (Matheny and Kingery, 2012). Anti-HAV immunoglobulin G (IgG) prophylaxis is effective in preventing clinical disease if it is given soon after exposure (Craig et al., 1998) but is unproven at post-exposure 14 days or later (WHO, 2012). IgG provides dose-dependent protection lasting up to five months (MMWR, 1996).

1.4.1 Vaccines

1.4.1.1 Types of vaccines

Since 1992 when the first inactivated vaccine against hepatitis A was developed, several licensed vaccines have become available and been used to control infections in humans. Although live attenuated vaccines require a single dose, they are not commonly used in vaccination programs where inactivated preparations are preferred despite requiring administration in a two-dose schedule. The inactivated vaccines contain the cell culture-adapted HAV strains, inactivated with formalin and adsorbed to aluminum hydroxide adjuvant or liposomes (virosomes) (Bovier, 2008). They are highly immunogenic, well tolerated, safe and offer effective long-lasting protection (Yoon et al., 2016).

1.4.1.2 Target populations

With the exception of infants younger than 12 months, children who are not immune to HAV infection should be vaccinated. Vaccination of population groups at higher risk of infection is recommended, for example childcare workers, persons exposed to sewage, injecting drug users, homosexual men and international travelers visiting regions of high HAV endemicity (Nothdurft, 2008). Immunocompromised individuals, hemophiliacs, elderly patients or persons with chronic liver disease regardless of the etiology should also receive the vaccine (Jeong and Lee, 2010). Vaccination of pregnant women should be based on the individual's risk of HAV exposure, as the vaccine's safety has not been confirmed. As most outbreaks, especially in areas of low endemicity, are associated with consumption of contaminated food or water, vaccination of workers in food-service establishments and particularly food handlers could decrease the numbers of food-related cases. Although vaccination of this target group would be beneficial, the costs of the vaccines are an obstacle to their common use in the food sector.

1.4.1.3 Vaccination strategies

Although a vaccination program is an effective strategy to control hepatitis A outbreaks, its broad use could be infeasible due to difficult logistics and the overall costs (Craig et al.,1998). Nevertheless, vaccination of people at risk (FitzSimons et al., 2010) or those living in countries with a relatively high proportion of the adult population susceptible to HAV (WHO, 2012) is still considered a cost-effective strategy for disease prevention. Inactivated vaccines are intended for the immunization of patients of all age groups starting from children above the age of 12 months. The first dose provides protective anti-HAV titers conferring short-term immunity, and the second dose given from 6 to 12 months later boosts the vaccination effect, significantly increasing the level of antibodies (Wu and Guo, 2013). The lowest level of protective anti-HAV

antibodies in patient sera is 20 mIU/ml, although in several studies levels as low as 10 mIU/ml were indicated as sufficient (Ott et al., 2012). The current evidence on the use of a single dose of inactivated hepatitis A vaccine shows that it generates levels of protective anti-HAV antibodies that can persist for almost 11 years with no need for a booster dose within at least 6 years (Ott and Wiersma, 2013). The protection could last even longer, for up to 15 years, when live attenuated vaccine is used (Ott et al., 2012).

1.4.1.4 Future aspects

Revision of the current vaccination schedule against hepatitis A and vaccine doses to achieve long-term protection is necessary to reduce the costs and to foster its more frequent use in the future. Furthermore, a constant monitoring of vaccinated populations is needed to evaluate the real long term persistence of virus-specific antibodies without the need for a booster dose. Also surveillance programs and follow-up monitoring of vaccinated individuals from different risk groups should be undertaken (are desirable) to provide additional data on vaccine safety and other effects on health.

1.4.2 Hygiene measures

Personal hygiene, adequate sanitation, and access to safe drinking water are key factors that prevent fecal-oral transmission of HAV (Franco et al., 2012). Precautions the same as those to avoid food poisoning should be taken with food and water and eating or drinking of contaminated food and water should be avoided. It should be taken into account that a higher risk of infection via consumption of contaminated food is also associated with imported food (Severi et al., 2015), especially from countries where HAV infections are frequently noted (Hutin et al., 1999).

Also good personal hygiene practices are important in preventing the infection. Proper hand washing by food handlers is an effective hygiene measure to reduce the risk of food contamination. For instance, hand washing with domestic or commercial topical agents or rinsing the hands with water alone could reduce virus transfer to food up to 30-fold (Bidawid et al., 2000). Although contamination of food could take place at different stages of the food supply chain, the most intensive food handling is during primary production. Therefore, adequate toilet and hand-washing facilities should be provided for field workers with the accompanying appropriate training regarding hygiene rules. Regardless of the stage of the food supply chain, food handlers with symptoms of infectious hepatitis should be excluded from work or from areas of food production or preparation and a medical check should precede their return to work. The water used for irrigation of food crops or shellfish farming should be free from microbial risk and microbiological safety guidelines should be followed wherever food is produced.

2.0 Environmental Occurrence, Persistence and Survival

2.1. Detection Methods

The identification of HAV in environmental samples is not an easy task, because often viruses are present in very low numbers, they are not distributed homogeneously in samples, and analytical procedures are far too complicated. Currently traditional virological methods (e.g. virus propagation in cell culture and electron microscopy) are hardly ever used for HAV detection due to low sensitivity or difficulties in propagation of wild types of HAV strains under laboratory conditions. In addition, the number of viruses in food or water is usually too low to be directly detected without prior concentration of the sample. Therefore, direct methods of viral analysis have gradually been replaced by matrix-specific procedures consisting of several consecutive steps, i.e. virus extraction and concentration followed by molecular detection (Cook et al., 2012). For water samples, there are several methods where virus concentration is performed by its adsorption to negatively or positively charged filters, glass powder or glass fiber (Bosch, 1998). For instance, filtration (Villar et al., 2007) or ultracentrifugation (Pina et al., 1998) are also successfully used for the recovery of viruses from wastewater samples. Apart from these methods, a new one based on tangential-flow ultrafiltration (Carducci et al., 2008; Hernandez-Morga et al., 2009) is also being employed. Removal of viruses from contaminated food surfaces can be achieved by mechanical treatment of the sample with subsequent concentration of viruses in a small volume by precipitation (Guévremont et al., 2006), ultracentrifugation (Rzeżutka et al., 2006) or immunomagnetic separation (IMS) (Suñén et al., 2004; Shan et al., 2005; Papafragkou et al., 2008).

In view of the type of samples tested, only the extraction and concentration steps are performed in different ways, whereas the virus detection step can be universal for different matrices. HAV detection can be performed using qualitative (RT-PCR, real-time RT-PCR) or quantitative (RT-qPCR) molecular methods. Despite vast numbers of published protocols utilising real-time technology, methods based on traditional PCR assays are still readily used, especially for virus detection and subsequent genotype identification in environmental samples (Nainan et al., 2006). Molecular methods mostly utilize selective amplification of cDNA fragments within the 5'-NCR region of the HAV genome, which was shown to be the most conserved among all HAV strains isolated to date (Costa-Mattioli et al., 2002; Abd El-Galil et al., 2005; Costafreda et al., 2006). As an alternative to these methods, a nucleic acid sequence-based amplification (NASBA) technique has been developed (Jean et al., 2001, 2002, 2004; Abd El-Galil et al., 2005). A novel method successfully employed in clinical virology is MALDI-TOF mass spectrometry. It allows for identification and characterization of viruses based on the detection of the mass of their molecules. Although the method has the potential to be broadly used in viral diagnostics it still requires further development in order to be employed in environmental studies (Cobo, 2013). The utility of metagenomic approaches for identification of enteroviruses in sewage sludge samples was recently demonstrated by Bibby and Peccia (2013).

2.2 Data on Occurrence

2.2.1 Raw and treated sewage

High abundance of HAV has been found in raw and treated sewage (Table 1). Virus remains in sewage after its treatment, as it was shown that up to 96% of treated sewage can contain HAV. The highest viral load detected in treated sewage was up to $3.8x10^5$ GC/L (Villar et al., 2007). Virus occurrence in sewage samples may reflect the epidemiology pattern of virus infections in the population (Pintó et al., 2007).

Table 1. Natural occurrence of HAV in sewage

Area	Study Period	Genotypes	Sample Type	Percent Positive (# of samples)	Concentration Average (GC/L)	Reference
Brazil (Florianópolis)	2007 to 2008	NR	Activated sludge	25% (3/12)	4.25E+05	Schlindwein et al., 2010
Brazil (Florianópolis)	2007 to 2008	NR	Wastewater Effluent (After Activated Sludge)	25% (3/12)	4.0E+05	Schlindwein et al., 2010
Brazil (Florianópolis)	2007 to 2008	NR	Raw Sewage	8.3% (1/12)	NR	Rigotto et al., 2010
Brazil (Rio de Janeiro)	2005	IA, IB	Raw sewage	88% (22/25)	5.1E+05	Villar et al., 2007
Brazil (Rio de Janeiro)	2005	IA, IB	Treated Sewage (After Activated Sludge)	96% (24/25)	2.95E+05	Villar et al., 2007
Brazil (Rio de Janeiro)	2009 to 2010	IA	Raw Sewage	58% (14/24)	3.7E+06	Prado et al., 2012
Brazil (Rio de Janeiro)	2005 to 2008	IA, IB	Raw Sewage	60% (3/5)	NR	Prado et al., 2011
Brazil (Rio de Janeiro)	2005 to 2008	IA	Raw Sewage	57% (4/7)	3.7E+07	Prado et al., 2011
Brazil (Rio de Janeiro)	2005 to 2008	IA	Treated Effluent (chlorination)	66.6% (4/6)	4.2E+07	Prado et al., 2011
Brazil (Limeira, Sao Paulo)	2004-2005	NR	Raw Sewage	48% (24/50)	NR	Medici Barrella et al., 2009
Egypt (Cairo)	1998 to 1999	IB	Raw sewage	71.4% (25/35)	NR	Pintó et al., 2007
Egypt (Cairo)	2006 to 2007	IB	Raw sewage	21% (8/38)	NR	Kamel et al., 2011
Greece (Patras)	2007 to 2009	IA	Raw sewage	8.3% (4/48)	NR	Kokkinos et al., 2011
Italy (Several regions)	2012 to 2013	IA, IB	Raw sewage	24.2% (38/157)	NR	La Rosa et al., 2014
Italy	2002 to 2003	IA, IB	Raw sewage	18.5% (5/27)	NR	Petrinca et al., 2009
Italy	2002 to 2003	IA, IB	Treated effluent (chlorinated)	9.1% (1/11)	NR	Petrinca et al., 2009

Area	Study Period	Genotypes	Sample Type	Percent Positive (# of samples)	Concentration Average (GC/L)	Reference
Singapore	2007	IA	Raw sewage	78% (14/18)	NR	Aw and Gin, 2010
Singapore	2007	IA	Secondary effluent	28% (5/18)	NR	Aw and Gin, 2010
Spain (Barcelona)	1998 to 2002	IB	Raw sewage	11.2% (28/249)	NR	Pintó et al., 2007
Tunisia (Kairouan, Sousse, Monastir)	2009 to 2010	IA, IB	Raw Sewage	66.9% (91/136)	3.7E+07	Ouardani et al., 2016
Tunisia (Kairouan, Sousse, Monastir)	2009 to 2010	IA, IB	Treated effluent (activated sludge)	40.7% (55/135)	9.8E+08	Ouardani et al., 2016

NR: Not Reported

2.2.2 Sludge

In treated activated sludge, HAV was detected in 25% of samples with the range of 3.1 to $5.4x10^5$ GC/Ll (Schlindwein et al., 2010).

2.2.3 Surface water

The virus prevalence in surface waters depends on the extent of human activities, the presence and size of the human settlements in close vicinity to the water courses and the environmental impact on areas that receive urban sewage (De Paula et al., 2007). The virus presence has been confirmed in different types of surface waters, eg. rivers (De Paula et al., 2007), lakes (Pusch et al., 2005) and estuarine waters (Hernandez-Morga et al., 2009) (Table 2). For instance, in river waters a 92% prevalence of the virus has been shown with quantities ranging from 60 to $5.5x10^3$ GC/L (De Paula et al., 2007).

Table 2. Natural occurrence of HAV in surface and ground waters

Area	Study Period	Genotypes	Sample Type	Percent Positive (# of Samples)	Concentrations Average Or Range (GC/L)	Reference
Brazil (Concórdia)	2011 to 2012	NR	Ground water	20% (1/5)	4.6E+05	Fongaro et al., 2015
Brazil (Florianópolis)	2011 to 2012	NR	Recreational river water	66.6% (4/6)	1.2E+05 to 2.03E+05	Fongaro et al., 2015
Brazil (Florianópolis)	2014	NR	Recreational water	27.1% (13/48)	1.1E+02 to 4.3E+03	Elmahdy et al., 2016
Brazil (Florianópolis)	2007 to 2008	NR	Recreational sea water	16.6% (2/12)	NR	Rigotto et al., 2010
Brazil (Florianópolis)	2007 to 2008	NR	Sea water	8.3% (2/24)	NR	Rigotto et al., 2010
Brazil (Florianópolis)	2007 to 2008	NR	Drinking water (untreated)	16.6% (2/12)	NR	Rigotto et al., 2010

Area	Study Period	Genotypes	Sample Type	Percent Positive (# of Samples)	Concentrations Average Or Range (GC/L)	Reference
Brazil (Manaus)	2004 to 2005	IA, IB	River water	92.3% (48/52)	60 to 5.5E+03	De Paula et al., 2007
China (Hong Kong)	NR	IB	Marine water	57.1% (4/7)	4.6E+02 to 1.03E+03	Yang et al., 2011
Korea (Geum/Seom, Han, Nakdong, Yeongsan)	2007 to 2010	IA, IB	River Water	3.4% (9/265)	NR	Lee et al., 2014
Mexico (Punta Bandera)	NR	NR	Ocean water	100% (4/4)	90 to 3.5E+03	Brooks et al., 2005
Mexico (Huizache Caimanero Lagunary Complex)	2006 to 2007	IB	Esturine water	80% (32/40)	NR	Hernandez-Morga et al., 2009
Mexico (Mazaltan, Atlanta Bay)	2005 to 2006	IB	Recreational marine water	9.4% (3/32)	NR	León Félix et al., 2010
Portugal (Lisbon coastal area)	2008	NR	Recreational ocean water	95.5% (21/22)	NR	Silva et al., 2010
South Africa (Polokwane)	2012	IB	River and Dam Waters	76.2% (16/21)	NR	Saïd et al., 2014
South Africa (Gauteng)	1997 to 2000	NR	Dam Water	14.9% (23/154)	8.53E+04	Venter et al., 2007
South Africa (Gauteng)	1997 to 2000	NR	River water	17.5% (27/154)	7.94E+03	Venter et al., 2007
USA (Imperial Beach pier, San Diego)	NR	NR	Ocean water	100% (4/4)	2.6E+03 to 3.5E+03	Brooks et al., 2005

NR: Not Reported

2.2.4 Groundwater

There have been several outbreaks reported in which drinking HAV sewage-contaminated well water led to human infection (Bloch et al., 1990; De Serres et al., 1999). This signifies that inadequacy in sewage disposal or storage greatly contributes to drinking water contamination. HAV showed its stability in untreated well water which was tested three months after the first case appeared. The concentrates of contaminated well water samples stored at 4°C for eight months still contained infectious virus (Bloch et al., 1990).

2.2.5 Drinking water

The occurrence of HAV in drinking water is not well documented. Nevertheless, the virus was sporadically detected in potable or spring water implicated in outbreaks of waterborne hepatitis A (Bosch et al., 1991; Tallon et al., 2008).

2.2.6 Seawater

The presence of HAV in coastal seawater samples has been demonstrated by Gersberg et al. (2006). Depending on the sampling area, the virus was detected in up to 86% of samples that contained from $9.51x10^2$ to $3.0771x10^4$ viral particles/L.

2.2.7 Soil

A 19.1% prevalence of HAV in soil has been reported by Parashar et al. (2011). The samples were collected from

sites located close to a sewage treatment plant.

2.2.8 Irrigation water and on crops

An HAV strain closely related to genotype V was detected in irrigation water, highlighting the importance of aquatic paths in virus transmission to food crops (Rachida et al., 2016). Although several outbreaks of hepatitis A were linked to green onions (Wheeler et al., 2005), semi-dried tomatoes (Petrignani et al., 2010) and frozen berries (Severi et al., 2015), the virus presence in the implicated foods has only been occasionally confirmed (Terio et al., 2015; Guzman-Herrador et al., 2015).

2.2.9 In shellfish

Human activities involving sewage outfall and illegal wastewater discharge contribute greatly to the contamination of the aquatic environment with enteric viruses (Maalouf et al., 2010; Williams-Woods et al., 2010). Bivalve molluscan shellfish are farmed in natural waters and therefore the quality of harvesting areas is constantly monitored for the presence of microbial contaminants. Nevertheless, there is still a high possibility of contamination of shellfish habitats with human enteric viruses. Hepatitis A is the most serious viral infection linked to shellfish consumption, and the largest-ever reported foodborne outbreak was due to HAV-contaminated shellfish (Lees, 2000). The occurrence of HAV in shellfish was shown not only in different cultured species but also in wild shellfish (Bigoraj et al., 2014). The virus prevalence ranged from 1.75% (Hansman et al., 2008) to 18.5% (Manso and Romalde, 2013). HAV subgenotypes detected in shellfish farmed in European coastal waters mirror the subgenotype distribution pattern of the virus strains detected in humans from the same region (Le Guyader et al., 2000).

2.2.10 In the air

Land application of biosolids constitutes a minimal risk of enterovirus infection from bioaerosols. In the studies conducted by Brooks et al., (2005) HAV RNA was not detected in bioaerosols generated during biosolid spreading, although the presence of other human enteric virus types was found.

2.3 Persistence

In comparison to other picornaviruses HAV is robust, being stable at lower pHs and temperatures up to 80°C (Wang et al., 2015). The lack of lipid envelope makes HAV resistant to many disinfectants which affect enveloped virus infectivity. For example, cell-adapted HAV survives in pH 1 when treated for two hours at room temperature, and virus infectivity is still retained after incubation of a virus suspension at 60°C for one hour. Infectivity can be preserved for at least one month after drying and storage at 25°C with 42% humidity or even for years at -20°C (Hollinger and Emerson, 2011). The virus loses its infectivity within seconds at 80°C, however when it is protected by proteinaceous matter a 4 $\log_{10}$ inactivation could be obtained at 85 to 90°C only after 1 min (Millard et al.,1987). Virus survival is facilitated by organic debris present in fecal matter (Kotwal and Cannon, 2014). HAV excreted in feces may be adsorbed or embedded in particles, which protects virus infectivity increasing the chance of environmental virus transmission. For example, fecally suspended HAV can survive for several hours on human hands or even for days on nonporous surfaces (Mbithi et al., 1991).

2.3.1 Surface water

There was little or no decay of HAV observed over a 48-day experimental period in river waters containing higher organic load. The prolonged persistence of the virus may have been in part due to the absence of indigenous microorganisms (Springthorpe et al.,1993).

2.3.2 Groundwater

The potential for HAV survival in sterile and non-sterile groundwater taken from a deep well was studied by Sobsey et al. (1986). The groundwater samples were seeded with HAV and incubated at 5°C and 25°C respectively. There was little inactivation of the virus at 5°C over the eight weeks, regardless of the sterility of the samples. Virus incubated at 25°C in sterile water survived without reduction in numbers over the eight weeks of the experiment, but in the non-sterile samples there was 2 $\log_{10}$ inactivation observed by the end of the second week.

2.3.3 Drinking water

In tap water samples stored at 4°C the titer of HAV decreased by 1.6 $\log_{10}$ after 55 days. The 99% reduction time was 56 days. By contrast however, at room temperature the titers of HAV declined by 4 and 3.5 $\log_{10}$ respectively after 3 and 50 days. The predicted 99% reduction time was 27 days (Enriquez et al., 1995). In bottled mineral water the virus persisted longest at 4°C with little inactivation during more than a year. However, at room temperature infectious virus was still detected after 300 days of exposure and this prolonged persistence would increase the risk to consumers following contamination of mineral water with enteric viruses (Biziagos et al., 1988).

2.3.4 Seawater

HAV-seeded seawater from Spanish coastal areas was incubated at 5°C and 25°C for 30 days. Little inactivation of virus was observed at 5°C but at 25°C virus decay was more pronounced (Bosch, 1995). However, in another experiment, coastal seawater samples from North Carolina, California and Hawaii were used. There was a 4 $\log_{10}$ decline reported in titer for HAV in about four weeks regardless of which seawater was tested (Callahan et al., 1995). Virus survival was dependent upon temperature, HAV declining more slowly at lower temperature, with an estimated 90% loss after 178 days at 25°C and 212 days at 19°C (Crance et al.,1998).

2.3.5 Food

HAV can persist in an infectious state in shellfish for more than three weeks and viral RNA can be still detected after six weeks following the contamination event (Kingsley and Richards, 2001). Virus persistence on fresh produce depends on temperature of storage, moisture, texture of the exposed surface and its adsorption capacity. Infectious virus was still detected on lettuce after nine days of storage at 4°C (Croci et al., 2002).

2.3.6 Biosolids

The type of treatment to which biosolids are subjected influences HAV survival. A 1 to 1.5 $\log_{10}$ of HAV RNA reduction in biosolids containing $FeCl_3$ used for phosphate control was observed after 60 days at 20°C. There was no significant virus loss seen at 4°C during the same storage time. In biosolids treated with lime and alum immediate inactivation of HAV was obtained. It was likely due to the alkaline pH of biosolids (Wei et al., 2010).

2.3.7 Soil

HAV survived for at least 12 weeks at 5°C in different types of soil suspensions. However, a 2 $\log_{10}$ inactivation rate was observed in soil incubated at 25°C for at least 8 weeks. Virus inactivation was less affected by temperature and microbial activity. Increased virus survival has been observed in clay soils, probably due to virus adsorption to clay providing greater resistance to inactivating environmental factors (Sobsey et al., 1986). A longer virus survival in soil under experimental conditions was reported by Parashar et al. (2011). HAV was relatively stable for 13 weeks at 37°C. The discrepancies in virus survival observed between these experiments could be due to the different types of soil used.

For example, survival of poliovirus type 1 has been studied in different soil types to which wastewater from a settled sewage sample containing virus was added. The soil materials used were represented by sand, clay, loam and a mixture of these materials. Virus survival depended on the type of soil material, with the highest (> 4.3 $\log_{10}$) inactivation rate observed after 34 days in soil containing sand, clay and loam. For all types of soil the achieved virus reduction was higher than 1.89 $\log_{10}$ (Sobsey et al., 1980).

3.0 Reduction by Sanitation Management

3.1 Wastewater Management

Studies that examine removal of HAV from wastewater subjected to a natural treatment process are scarce. In addition, none of these studies examined the behavior or fate of HAV present in anthropogenic discharge. Nevertheless, some data exist for poliovirus which similar to HAV is also a picornavirus. Therefore, information gathered on the survival of this virus type could give an overview on transport and removal of HAV during wastewater treatment.

3.1.1 Water-based sanitation – onsite

Storage of human waste at 5°C in the form of septic tank effluent allowed 1 $\log_{10}$ reduction of HAV titer to be achieved in 58.5 days. Only 35.1 days were required to obtain the same level of virus reduction if wastes were stored at 22°C. At the same storage temperature HAV was inactivated rapidly within 17.1 days in mixtures of human wastes and animal manure. This observation indicates antiviral activity of bacterial microflora present in manure and its important role in virus inactivation (Deng and Cliver, 1995).

3.1.2 Wastewater stabilization ponds

Attenuated poliovirus 1 (LSC) strain survived 5 days' treatment in an experimental oxidation pond with no decrease in virus titer (Kott et al., 1974). This suggests that human enteric viruses may be found in wastewater effluents and further treatment is necessary to inactivate them. Chlorination of oxidation pond effluent is an effective virus removal treatment. For example, sewage retention in oxidation ponds for 18 days with subsequent effluent chlorination efficiently removed poliovirus from sewage, as none of the tested effluent samples contained viruses (Vithalbhai et al., 1982).

3.1.3 Wetlands

Constructed wetlands have been shown to efficiently reduce human bacterial pathogens (Karim et al., 2008) and enteroviruses (Rachmadi et al., 2014) in wastewater. Studies on removal of poliovirus type 1 from artificially constructed vegetated wetlands showed that poliovirus inactivation in wetlands receiving fresh water was greater than in wetlands with sewage influx (Karim et al., 2008).

3.1.4 Wastewater treatment facilities

3.1.4.1 Secondary treatment: activated sludge

Municipal sewage after primary (sedimentation) and secondary (activated sludge) treatment can contain HAV. A high HAV stability in the final effluent from an activated sludge treatment plant was demonstrated and difficulty in its removal by conventional sewage treatments (Morace et al., 2002).

3.1.4.2 Secondary treatment: anaerobic digestion

Low HAV abundance in sludge after mesophilic anaerobic digestion has been demonstrated by Bibby and Peccia (2013). The virus presence was not confirmed by metagenomic analyses in any of the samples tested, although a vast range of environmentally less resistant DNA and RNA viruses than HAV were identified. Biosolids obtained after treatment of the sludge by dewatering or mesophilic anaerobic digestion should be considered HAV-free despite the common presence of other enteric viruses (Wong et al., 2010).

3.1.4.3 Biosolids treatment

Several factors such as temperature, moisture, presence of indigenous microflora, pH, sunlight, oxygen and soil type influence inactivation of the pathogens in

biosolids (Sidhu and Toze, 2009). Alkaline stabilization of sludge at 28°C appeared to be an efficient HAV-removal process. The virus at an initial titer of 6 $\log_{10}$ PFU/ml was not detected after two hours. When sludge treatment was conducted at 4°C, the same level of virus reduction was achieved after 24 hours (Katz and Margolin, 2007).

3.2 Disinfection

3.2.1 Chlorination

Chlorination is widely used as a method of wastewater treatment. It was shown to be an effective disinfectant against poliovirus seeded into primary treated wastewater. Chlorine at a dose of 30 mg/L was sufficient to inactivate the virus within 15 min. However, the virucidal effectiveness of chlorination was dose dependent and virus sensitivity or resistance to free chlorine is also associated with suspended solids shielding the virus particles against disinfectant action (Tree et al., 2003).

3.2.2 Ultraviolet

UV light is effective in removal of a broad range of pathogenic microbes present in water (McGuigan et al., 2012). Under simulated sunlight conditions (850 W m^{-2}) as a means of disinfection, poliovirus was completely inactivated in water at 40°C after four hours, with a total 4.3 $\log_{10}$ reduction of the virus titer. A longer disinfection time (six hours) was required when the water temperature was 25°C (Heaselgrave et al., 2006) When protected from sunlight less inactivation was observed at both temperatures. HAV is assumed to be similar to poliovirus in this regard.

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