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Hepatitis A Virus: State of the Art

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Abstract Hepatitis A is the most common among all hepatitis worldwide in spite of an efficient vaccine and improved hygiene. Shellfish-borne outbreaks are still of major concern causing hundreds of cases and huge economical losses in the present context of global food trade. Hepatitis A virus (HAV) is a unique picornavirus with many differences in its molecular biology including both its incapacity to induce the inhibition of the cellular protein synthesis and a highly biased and deoptimized codon usage with respect the cell. The final goal of this intriguing strategy seems to be the need for a fine-tuning control of the translation kinetics, particularly at the capsid coding region, and the underlying mechanism is the use of a right combination of common and rare codons to allow a regulated ribosome traffic rate thus ensuring the proper protein folding. Capsid folding is critical to warrant a high environmental stability for a virus transmitted through the fecal–oral route with long extracorporeal periods.

Keywords Hepatitis A · Picornavirus · Shellfish · Bivalves · Food-borne outbreaks

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Introduction

Four hundred years b.c., Hippocrates described an illness characterized by episodes of jaundice that could probably correspond to a viral hepatitis. Two thousand three hundred years later, at the beginning of the twentieth century, the term "infectious hepatitis" was defined and associated to a kind of infectious jaundice occurring in epidemics. In the early 1940s two separate entities were identified "infectious" and "serum" hepatitis, and from 1965 to nowadays the major etiological agents (hepatitis A, B, C, D and E viruses) of viral hepatitis have been identified. While all viral hepatitis are infectious the previously "infectious" and "serum" terms refer to the mode of transmission. The "infectious" type corresponds to those hepatitis transmitted through the fecal–oral route, or enteric hepatitis, and the "serum" hepatitis to those parenterally transmitted. The enteric hepatitis includes two types: hepatitis A and E which can be food borne and waterborne. The present chapter will focus on both the disease and the etiological agent of hepatitis A.

Hepatitis A Infection: Natural Course and Epidemiology

Hepatitis A infection mostly develops asymptomatically or subclinically among young children (under 5), while in older children and in the adulthood the infection usually proceeds with symptoms (Previsani et al. 2004). In this latter case, the clinical course of hepatitis A is indistinguishable from that of other types of acute viral hepatitis. The clinical case definition for hepatitis A is an acute illness with moderate onset of symptoms (fever, malaise, anorexia, nausea, abdominal discomfort, dark urine) and jaundice,

and elevated serum bilirubin and aminotransferases levels later on. The incubation period of hepatitis A ranges from 15 to 50 days and clinical illness usually does not last longer than 2 months, although 10–15% of patients have prolonged or relapsing signs and symptoms for up to 6 months (Glikson et al. 1992; Sjogren et al. 1987). In fact, with the advent of new highly sensitive techniques even in normal clinical courses a high and long lasting viremia has been detected (Costafreda et al. 2006), with the peak (up to 10^7 genome copies/ml of sera) occurring at 2 weeks after the onset of symptoms and lasting up to an average of 6 weeks after the start of symptoms (Bower et al. 2000; Costafreda et al. 2006). There is no evidence of chronicity of the infection, however, occasionally the infection may proceed to a fulminant hepatitis, mainly among patients with underlying chronic liver diseases (Akriviadis and Redeker 1989; Previsani et al. 2004).

The distribution patterns of hepatitis A in different geographical areas of the world are closely related to their socioeconomic development (Gust 1992; Hollinger and Emerson 2007; Previsani et al. 2004). The endemicity is low in developed regions and high in underdeveloped countries. The epidemiological pattern has important implications on the average age of exposure and hence, as above stated, on the severity of the clinical disease. Since hepatitis A infection induces a life-long immunity (Hollinger and Emerson 2007), severe infections among adults are rare in highly endemic regions where most children are infected early in life. In contrast, in low endemic areas the disease occurs mostly in adulthood, mainly as a consequence of traveling to endemic regions, having sexual risky practices or consuming contaminated water or food and hence the likelihood of developing severe symptomatic illness is high. An epidemiological shift, from intermediate to low prevalence, has been noticed in recent decades in many countries, particularly in Southern Europe, including Spain, Italy, and Greece (Domínguez et al. 2008; Germinario et al. 2000; Van Damme and Van Herck 2005). Consequently, the Mediterranean basin as a whole should no longer be considered as an endemic area (Pintó et al. 2007a; Previsani et al. 2004).

Additionally, some other countries from Eastern Europe (Cianciara 2000; Tallo et al. 2003) have also described significant declines in the incidence of hepatitis A. Likewise, in several Asian and American countries a shift from highly to moderate endemic has as well been described (Barzaga 2000; Tanaka 2000).

Hepatitis A Transmission: Shellfish as a Source of Big Outbreaks

After replication in the liver, hepatitis A virus (HAV) is found in the bile in large quantities, reaching the intestines by the bile duct, and being subsequently shed in feces. Virion stability of HAV in the presence of biliary salts is guaranteed by the absence of a lipid envelope, which is not the case for serum hepatitis viruses. Symptomatic individuals as well as asymptomatic carriers shed virus that may contaminate water and food. HAV concentration in the patient stools is highest (up to 10^{11} genome copies/g of feces) after 2 weeks of the onset of symptoms and lasts at least four more weeks. An additional concern is that viral excretion even in symptomatic patients starts before the onset of symptoms. Hepatitis A infection is mainly propagated via the fecal–oral route being the person-to-person contact the most common mode of transmission. In fact HAV persistence in contaminated fomites, such as sanitary paper, sanitary tile, and latex gloves, is very long (Abad et al. 1994a). In consequence, given the high excretion level of HAV, transmission of the infection is facilitated when poor sanitary conditions occur. In addition, active homosexual men are a risk group for HAV transmission and outbreaks are frequently reported (Stene-Johansen et al. 2002, 2007; Tortajada et al. 2009). Transmission through the parental route may also occasionally occur (Noble et al. 1984; Sheretz et al. 2005).

Viruses present in the stool of infected patients are discharged into sewage which ultimately may contaminate surface waters and seawater, and consequently be acquired and concentrated by shellfish growing in these waters. While in approximately 40% of the reported cases of hepatitis A the source of infection cannot be identified, food-borne outbreaks of the disease have been reported. Within this latter category, shellfish grown and harvested from waters receiving urban contaminants is a cause of many outbreaks of infectious hepatitis. The first documented shellfish-borne outbreak of "infectious hepatitis" occurred in Sweden in 1955, when 629 cases were associated with raw oyster consumption (Roos 1956). However, the most significant outbreak of HAV infection occurred in Shanghai, China, in 1988, in which almost 300,000 cases were caused by consumption of clams harvested from a sewage-polluted area (Halliday et al. 1991). In fact, this is so far the largest virus-associated outbreak of food poisoning ever reported. Smaller outbreaks have been reported worldwide (Conaty et al. 2000; Leoni et al. 1998; Mackowiak et al. 1976; Mele et al. 1989; Pintó et al. 2009; Sánchez et al. 2002; Stroffolini et al. 1990). Several issues, beside environmental parameters (see Maalouf same issue), such as the fact that fecal HAV excretion precedes the onset of symptoms, together with the difficulties to completely remove and or inactivate viruses through sewage treatment (Blatchley III et al. 2007; Bosch 2007) makes hepatitis shellfish-borne outbreaks hard to prevent if the virus is circulating among the population.

Hepatitis A Virus: General Features

The etiological agent of hepatitis A is the HAV which belongs to genus *Hepatovirus* within family Picornaviridae, and as such it consists of a non-enveloped icosahedral capsid of around 30 nm in diameter containing a positive ssRNA genomic molecule of 7.5 kb (Fauquet et al. 2005). The genome contains a single open reading frame (ORF) encoding a polyprotein of around 2225 amino acids preceded by a 5′ non-coding-region (5′NCR) that makes around 10% of the total genome, and followed by a much shorter 3′NCR that contains a poly(A) tract (Baroudy et al. 1985; Cohen et al. 1987). This genome is uncapped but covalently linked to a small viral protein (VPg) (Weitz et al. 1986). The singly translated polyprotein is subsequently cleaved into 11 proteins through a cascade of proteolytic events brought about mainly by the viral 3C protease (Schultheiss et al. 1994; Schultheiss et al. 1995). A general scheme of HAV genome organization and translation products is depicted in Fig. 1. However, although the general genomic organization and the expression pattern of HAV are very similar to those of most picornaviruses (Agol 2002; Hollinger and Emerson 2007), many differences exist which are discussed in the next section.

HAV Unique Properties: The Low Translation Rate Necessary to Ensure a High Capsid Stability is Associated with a Low Antigenic Variability

The genetic distance between the genus *Hepatovirus* and the other genera of the family reflects not merely a difference in the nucleotide and amino acid composition but a difference in the molecular and biological characteristics of HAV. From the genomic and proteomic points of view, several interrelated key issues must be brought up. First of all, the structure of the 5′NCR and its internal ribosome entry site (IRES). The HAV IRES is unique among picornaviruses and constitutes the type III model (Brown et al. 1994; Ehrenfeld and Teterina 2002), which shows a very low efficiency in directing translation (Whetter et al. 1994) compared to the other picornavirus IRESs. Second, HAV encodes only a protease, 3C, while other picornavirus codes for additional proteases such as the L protease, in genus *Aphtovirus*, or the 2A protease in *Enterovirus* and *Rhinovirus* genera (Leong et al. 2002). L and 2A proteases, when present, play a crucial role in the primary cleavages of the viral polyprotein while in those genera lacking these proteases, such *Hepatovirus* and *Paraechovirus*, both primary and secondary cleavages are conducted by the 3C protease. But what is most important is that these additional proteases are involved in the cellular protein shutoff induction (Leong et al. 2002). Since picornaviruses utilize a mechanism of translation that is cap-independent and IRES-dependent, the inhibition of non-essential cap-dependent cellular translation could be advantageous to the virus. In doing so, the cellular translation machinery is utilized almost exclusively for the production of viral proteins (Kuechler et al. 2002). An early event preceding the shutoff of host cell protein synthesis is the cleavage of the cellular translation initiation factor eIF4G, and evidence exists supporting that the enzymes responsible of such a cleavage are 2A and L proteases in enteroviruses and rhinoviruses, and aphtoviruses, respectively (Kuechler et al. 2002). An immediate consequence of the lack of any of these proteolytic activities in HAV is its incapacity to induce cellular shutoff which otherwise is directly related with its requirement for an intact uncleaved eIF4G factor for the formation of the initiation of translation complex

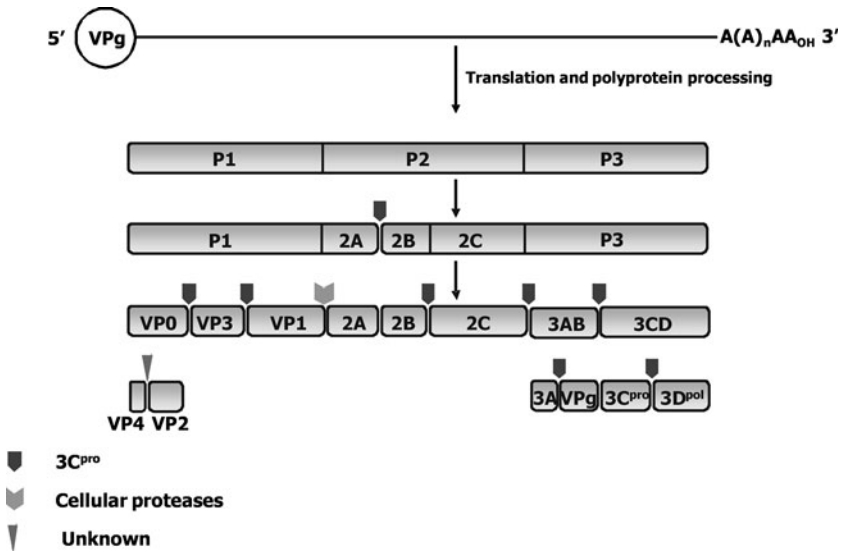

Fig. 1 Organization of the hepatitis A virus genome. *Top* Diagram of the virus genome depicting the genome-linked VPg protein at the 5′ end and the poly A tail at the 3′ end. *Bottom* Proteolytic processing pattern of the polyprotein resulting after translation

(Borman et al. 1997; Jackson 2002). What has been described up to now denotes that HAV must inefficiently compete for the cellular translational machinery and thus it presents a unique translation strategy. This points out to the third difference between HAV and other picornavirus members: the codon usage. HAV presents a higher codon usage bias compared to other members of its family, which conveys in the adaptation to use abundant and rare codons (Sánchez et al. 2003b). In fact, 14 amino acid codon families contain rare codons, defined in terms of their frequencies, making a total of 22 used rare codons (Pintó et al. 2007b). But what is more surprising is that the HAV codon usage has evolved to be complementary to that of human cells, never adopting as abundant codons those abundant for the host cell, and even in some instances using these latter as rare codons. This disparity, unique to HAV, has been interpreted as a subtle strategy to avoid, as much as possible, competition for the cellular tRNAs in the absence of a precise mechanism of inducing shut-off of cellular protein synthesis (Sánchez et al. 2003b). As stated before, a consequence of this special codon bias is an increase in the number of rare codons used by HAV. Overall this increment is the result of the addition to the cellular rare codons, also used as rare by the virus, of those most abundant cellular codons that being unavailable for the virus are used at low frequencies. Altogether, the HAV codon usage may contribute to its slow replication and to its low yields. It has been largely documented (Chou and Lakatos 2004; Robinson et al. 1984; Sorensen et al. 1989) the role of rare codons in the control of translation speed, in the sense that clusters of rare codons would induce a transient stop of the translational complex in order to seek for a suitable tRNA present at a very low concentration among the pool of tRNAs. A function of these ribosome stallings has been suggested to be the assurance of the proper folding of the nascent protein (Adzhubei et al. 1996; Evans et al. 2005; Gavrilin et al. 2000). Such a function has been postulated for HAV, where highly conserved clusters of rare codons strategically located at the carboxy-ends of the structured elements of the capsid coding region have been reported (Sánchez et al. 2003b). In fact, the critical role of HAV codon usage, and particularly of these clusters of rare codons of the capsid coding region, has been shown during the process of adaptation of HAV to conditions of artificially induced cellular shutoff (Aragonès et al 2010). An overall change in codon usage was necessary to regain viral fitness in these conditions, with a clear re-deoptimization with respect to the cellular codon usage, and particularly affecting the rare codons located at the abovementioned strategic positions of the capsid (Aragonès et al 2010). This mechanism of adaptation to the cellular shutoff proves translation kinetics, i.e., the right combination of codons (common and rare) that allows a regulated ribosome traffic rate ensuring the proper protein folding, as the driving selective force of HAV codon usage.

Additionally, a certain contribution of the codon usage to the low variability of the HAV capsid has been proposed taking into account that 15% of its surface residues are encoded by such functional rare codons, (Sánchez et al. 2003b). This low capsid variability indeed correlates with a very low antigenic variability: a single serotype exists, being this another striking difference with other picornaviruses. The low capsid variability relies on negative selection acting against newly arising proteins with replacements affecting the residues encoded by rare codons of the capsid surface, even under immune pressure (Aragonès et al. 2008), confirming again certain beneficial role of such rare codons. Since the clusters of rare codons are located near or at the epitope regions, the need to maintain such clusters might prevent the emergence of new serotypes.

Other important differences exist between HAV and other picornaviruses at the morphogenetic/structural level. The role of both ends (amino-VP4 and carboxi-2A) of the capsid polyprotein in the virion assembly is still controversial (Probst et al. 1999), and while there is no agreement on the requirement of VP4 for the maturation of pentamers into capsids, a complete consensus exists on the necessity of 2A for pentamer formation (Martin and Lemon 2006; Probst et al. 1999). The ulterior removal of 2A in the mature virion must be performed by a host cell protease (Graff et al. 1999; Martin et al. 1999), although the mature 2A protein has never been identified directly in infected cells.

The X-ray crystallographic structure has not yet been solved, due to the low viral yields obtained by in vitro replication. However, recent three-dimensional images of HAV produced by cryoelectron microscopy (Holland Cheng, unpublished results) have revealed important data being the most intriguing the lack of a well-defined canyon around the fivefold axis of symmetry. The pit region of many picornaviruses contains the receptor binding residues playing, thus, an important biological role. A human HAV receptor (huhavr-1) has been identified (Feigelstock et al. 1998), which contains both an amino terminal Ig-like domain followed by a mucin-like domain (Silberstein et al. 2003). Huhavr-1 has been detected in several human tissues, including the liver. Alternatively, the asialoglycoprotein receptor, to which IgA binds to, has been described to enable HAV internalization provided that the virus is complexed with such immunoglobulin (Dotzauer et al. 2000). However, whichever is the receptor, the capsid region involved in such an interaction remains to be elucidated. In contrast, the capsid region interacting with the glycophorin A of the human erythrocytes is indeed located around the putative pit area (Sánchez et al. 2004). The capsid structure, however, is such that tolerates this

interaction only to occur at acid conditions, being impaired at neutral biological conditions. Erythrocyte glycoproteins may function as decoy receptors attracting pathogens to the erythrocyte and keeping them away from target tissues (Gagneux and Varki 1999), and hence the actual capsid conformation that allows escaping from erythrocyte attachment may constitute an advantage for a viremic infectious agent whose target organ is the liver. In fact, pathogenesis is in part determined by the spread of the virus to the target tissues. In this context, key factors for the viral biological cycle and infection outcome are a high stability to the acid pH of the stomach during the entry phase, a safe viremic phase, and resistance to the action of detergents, particularly biliary salts, during the exit phase. This extremely resistant phenotype of HAV explains its high persistence in the environment (Abad et al. 1994a, b) and its transmission by contaminated foods and drinking water (Bosch et al. 1991; Dentinger et al. 2001; Pintó et al. 2009; Reid and Robinson 1987; Rosemblum et al. 1990; Sánchez et al. 2002), which probably are the result of a highly cohesive capsid conformation mediated through a very accurate folding ensured by a highly controlled kinetics of translation.

Quasispecies and its Implications in Genetic Variability

Viral genetic variability results from the universal mechanisms of mutation, recombination, and genome segment reassortment, all them being replication-dependent. Since virus populations replicate at exceptionally high rates, they may be extremely variable. All this is particularly critical in RNA viruses, since they rely on error-prone polymerases lacking proof-reading activity which leads to complex mutant genome populations or quasispecies. Viral quasispecies are dynamic distributions of nonidentical but closely related viral genomes subjected to a continuous process of genetic variation, competition, and selection and which act as a unit of selection (review in Domingo et al. 2006). HAV as a RNA virus occurs as a swarm of mutants termed quasispecies (Sánchez et al. 2003a). In spite of the low antigenic variability of HAV, a certain degree of nucleotide variability, similar to that of other picornavirus, exists and allows the differentiation of HAV into several genotypes and subgenotypes. Different genomic regions, mainly from the capsid coding region (P1) or the junction between the capsid region (P1) and the contiguous non-structural region (P2), have been used to differentiate the genotypes. Particularly, the carboxy-terminus of the VP3 structural protein, the amino-terminus of the VP1 structural protein, the VP1X2A junction, the region spanning the carboxy-end of VP1 till the amino-terminus of 2B (VP1/P2B), and finally the entire VP1 region (see the review of Nainan et al. 2006). However, partial genomic sequences will never guarantee the reliability of the complete P1/2A region. As a matter of fact, the identification of some HAV antigenic variants affecting residues not included in the genotyping regions (Costa-Mattioli et al. 2002; Gabrieli et al. 2004; Sánchez et al. 2002) could have been elusive in such circumstances. This is the reason why the use of long genomic regions covering at least the entire VP1 including its 2A junction, has recently been recommended (Costa-Mattioli et al. 2002); European HAV Network, unpublished results) for a more broad molecular typing of HAV. Nevertheless, the VP1X2A junction is still the genomic region most in use worldwide (Robertson et al. 1992). In this region, seven genotypes were initially defined, whose genetic distance was >15% nucleotide variation. After refining this classification through the addition of more sequences, only six genotypes exist at the present time (Costa-Mattioli et al. 2002; Lu et al. 2004). Three out of these six genotypes (I, II, and III) are of human origin while the others (IV, V, and VI) are of simian origin. Genotypes I and II contain subgenotypes (Ia, Ib, IIa, and IIb) defined by a nucleotide divergence of 7–7.5%. Although it is generally accepted that the severity of hepatitis A is mostly related with host factors such as aging and the occurrence of other underlying liver diseases, viral factors may also play a role in pathogenesis. Among these viral factors it may be pointed that some mutations at the 5′NCR of HAV or at the VP1X2A and 2C regions have been associated with fulminant hepatitis (Fujiwara et al. 2001, 2002, 2003) or higher virulence in tamarinds (Emerson et al. 2002), respectively. However, there is no consensus whether the VP1X2A-derived genotypes are clinically different, although some strains belonging to the former genotype VII now included in genotype II were associated with fulminant cases (Ching et al. 2002; Costa-Mattioli et al. 2002).

In addition to the clinical implications of genetic variability, genotype characterization may be highly relevant to trace the origin of outbreaks. However, when typing outbreak-related isolates, it must be borne in mind that not always an identical nucleotide sequence is obtained from a putative source virus (e.g. contaminated food or water) and the virus found in the infected recipients. High mutation rates render very unlikely the complete conservation of sequences as soon as virus replication occurs, in this case in the infected individuals.

Prevention of Hepatitis A

Inactivated HAV vaccines are available since the early 1990s and provide long-lasting immunity against hepatitis A infection. The immunity is largely related to the induction of high titers of specific antibodies. Thanks to the

existence of a single serotype of HAV, these vaccines are of high efficacy. These vaccines consist of viruses grown in cell culture, purified, inactivated with formalin and adsorbed to an aluminum hydroxide adjuvant, making their economic cost quite high. This is the reason why many discrepancies already exist on their universal use in massive vaccination campaigns. Countries with previous intermediate endemicity of HAV such as Israel or some Autonomous Communities of Spain such as Catalonia, or some States of United States have performed studies on the impact of child vaccination on the overall incidence of hepatitis A concluding that the immunization is medically (Domínguez et al. 2008; Wasley et al. 2005) and economically (Dagan et al. 2005) justified. In contrast other countries in a similar situation such as Italy do not recommend at present the implementation of such a measure in terms of cost-benefits (Franco and Vitiello 2003). In this context is quite evident that high endemic countries that usually have low economic incomes do not regard the vaccination against hepatitis A as a primary policy (Teppakdee et al. 2002).

Although several attenuated vaccine candidates have also been attempted, due to the successful use of inactivated vaccines, its development is hardly plausible.

As a general rule in low and intermediate endemic regions, where paradoxically the severity of the disease is high, vaccination against hepatitis A should be recommended in high-risk groups, including travelers to high endemic areas, men having sex with men, drug users and patients receiving blood products. In addition, the inclusion of hepatitis A vaccines in mass vaccination programs in those countries receiving high numbers of immigrants from endemic countries is particularly advisable. However, and bearing in mind the quasiespecies replication pattern of HAV (Sánchez et al. 2003a) that in populations with continued exposure to the virus could lead to the selection of new antigenic variants escaping immune protection (Aragonès et al. 2008), mass vaccination programs in highly endemic areas are controversial.

Food safety implementation programs represent important prevention actions. Prospective virological analysis of shellfish is such a highly complex and costly process that obviously cannot be universally applicable to the huge production of shellfish. The first difficulty to overcome is to choose representative samples; even more when the screening procedures are based on molecular analysis of minute amounts of a sample, whose virus contamination, if present, is very low. A sensible prospective food safety approach is to identify and prevent hazards that could cause food-borne illnesses, rather than relying on spot-checks of manufacturing processes and random sampling of finished products to ensure safety (Pintó and Bosch 2008). For instance, in the particular case of hepatitis A, significant correlation between cases in the harvesting areas and positive HAV isolation in clams has been observed (Pintó et al. 2009). However, when evidence shows that a critical limit of viral contamination has been exceeded in the potential sources of contamination discharging into the shellfish growing beds, quantitative virological analysis addressing QA/QC requirements should be performed in the bivalves, since HAV levels in shellfish are predictors of the magnitude of the outbreak and can be used for risk assessment purposes (Pintó et al. 2009).

Future Impact

Many centuries after Hippocrates description of hepatitis clinical symptoms, HAV is still the most common cause of viral hepatitis worldwide in spite of an efficient vaccine and improved hygiene that do not prevent the occurrence of shellfish-borne outbreaks. Global trade, climate change, and the inherent genetic variability in association with vaccination campaigns may promote the emergence of new variants. All this calls for the need to trace and characterize the circulating strains.

References

Abad, F. X., Pintó, R. M., & Bosch, A. (1994a). Survival of enteric viruses on environmental fomites. *Applied and Environmental Microbiology, 60*, 3704–3710.

Abad, F. X., Pintó, R. M., Díez, J. M., & Bosch, A. (1994b). Disinfection of human enteric viruses in water by copper and silver in combination with low levels of chlorine. *Applied and Environmental Microbiology, 60*, 2377–2383.

Adzhubei, A. A., Adzhubei, I. A., Krasheninnikov, I. A., & Neidle, S. (1996). Non-random usage of "degenerate" codons is related to protein three-dimensional structure. *FEBS Letters, 399*, 78–82.

Agol, V. I. (2002). Picornavirus genetics: An overview. In B. L. A. W. E. Semler (Ed.), *Molecular biology of picornaviruses* (pp. 269–284). Washington, DC: ASM.

Akriviadis, E. A., & Redeker, A. G. (1989). Fulminant hepatitis A in intravenous drug users with chronic liver disease. *Annals of Internal Medicine, 110*, 838–839.

Aragonès, L., Bosch, A., & Pintó, R. M. (2008). Hepatitis A virus mutant spectra under the selective pressure of monoclonal antibodies: Codon usage constraints limit capsid variability. *Journal of Virology, 82*, 1688–1700.

Aragonès, L., Guix, S., Ribes, E., Bosch, A., & Pintó, R. M. (2010). Fine-tuning translation kinetics selection as the driving force of codon usage bias in the hepatitis A virus capsid. *PLoS Pathogens, 6*, e1000797.

Baroudy, B. M., Ticehurst, J. R., Miele, T. A., Maizel, J. V., Jr., Purcell, R. H., & Feinstone, S. M. (1985). Sequence analysis of hepatitis A virus cDNA coding for capsid proteins and RNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America, 82*, 2143–2147.

Barzaga, N. G. (2000). Hepatitis A shifting epidemiology in South-East Asia and China. *Vaccine, 18*, S61–S64.

Blatchley, E. R., III, Gong, W. L., Alleman, J. E., Rose, J. B., Huffman, D. E., Otaki, M., et al. (2007). Effects of wastewater disinfection on waterborne bacteria and viruses. *Water Environmental Research, 79*, 81–92.

Borman, A. M., Kirchweger, R., Ziegler, E., Rhoads, R. E., Skern, T., & Kean, K. M. (1997). elF4G and its proteolytic cleavage products: Effect on initiation of protein synthesis from capped, uncapped, and IRES-containing mRNAs. *RNA, 3*, 186–196.

Bosch, A. (2007). *Human viruses in water*. Amsterdam, The Netherlands: Elsevier.

Bosch, A., Lucena, F., Díez, J. M., Gajardo, R., & Blasi, M. (1991). Waterborne viruses associated with hepatitis outbreak. *Journal of the American Water Works Association, 83*(3), 80–83.

Bower, W., Nainan, O., Han, X., & Margolis, H. (2000). Duration of viremia in hepatitis A virus infection. *Journal of Infectious Diseases, 182*, 12–17.

Brown, E. A., Zajac, A. J., & Lemon, S. M. (1994). In vitro characterization of an internal ribosomal entry site (IRES) present within the 5′ nontranslated region of hepatitis A virus RNA: Comparison with the IRES of encephalomyocarditis virus. *Journal of Virology, 68*, 1066–1074.

Ching, K. Z., Nakano, T., Chapman, L. E., Demby, A., & Robertson, B. H. (2002). Genetic characterization of wild-type genotype VII hepatitis A virus. *Journal of General Virology, 83*, 53–60.

Chou, T., & Lakatos, G. (2004). Clustered bottlenecks in mRNA translation and protein synthesis. *Physical Review Letters, 93*, 198101–198104.

Cianciara, J. (2000). Hepatitis A shifting epidemiology in Poland and Eastern Europe. *Vaccine, 18*(Suppl 1), S68–S70.

Cohen, J. I., Ticehurst, J. R., Purcell, R. H., Buckler-White, A., & Baroudy, B. M. (1987). Complete nucleotide sequence of wild-type hepatitis A virus: Comparison with different strains of hepatitis A virus and other picornaviruses. *Journal of Virology, 61*, 50–59.

Conaty, S., Bird, P., Bell, G., Kraa, E., Grohmann, G., & McAnulty, J. M. (2000). Hepatitis A in New South Wales, Australia from consumption of oysters: The first reported outbreak. *Epidemiology and Infection, 124*, 121–130.

Costafreda, M. I., Bosch, A., & Pintó, R. M. (2006). Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. *Applied and Environmental Microbiology, 72*, 3846–3855.

Costa-Mattioli, M., Cristina, J., Romero, H., Pérez-Bercof, R., Casane, D., Colina, R., et al. (2002). Molecular evolution of hepatitis A virus: A new classification based on the complete VP1 protein. *Journal of Virology, 76*, 9516–9525.

Dagan, R., Leventhal, A., Anis, E., Slater, P., Ashur, Y., & Shouval, D. (2005). Incidence of hepatitis A in Israel following universal immunization of toddlers. *JAMA-Journal of the American Medical Association, 294*, 202–210.

Dentinger, C. M., Bower, W. A., Nainan, O. V., Cotter, S. M., Myers, G., Dubusky, L. M., et al. (2001). An outbreak of hepatitis A associated with green onions. *Journal of Infectious Diseases, 183*, 1273–1276.

Domingo, E., Martin, V., Perales, C., Grande-Pérez, A., Garcia-Arriaza, J., & Arias, A. (2006). Viruses as quasispecies: Biological implications. *Current Topics in Microbiology and Immunology, 299*, 51–82.

Domínguez, A., Oviedo, M., Carmona, G., Batalla, J., Bruguera, M., Salleras, L., et al. (2008). Impact and effectiveness of a mass hepatitis A vaccination programme of preadolescents seven years after introduction. *Vaccine, 26*, 1737–1741.

Dotzauer, A., Gebhardt, U., Bieback, K., Gottke, U., Kracke, A., Mages, J., et al. (2000). Hepatitis A virus-specific immunoglobulin A mediates infection of hepatocytes with hepatitis A virus via the asialoglycoprotein receptor. *Journal of Virology, 74*, 10950–10957.

Ehrenfeld, E., & Teterina, N. L. (2002). Initiation of translation of picornavirus RNAs: Structure and function of the internal ribosome entry site. In B. L. W. E. Semler (Ed.), *Molecular biology of picornaviruses* (pp. 159–169). Washington, DC: ASM Press.

Emerson, S. U., Huang, Y. K., Nguyen, H., Brockington, A., Govindarajan, S., St, C. M., et al. (2002). Identification of VP1/2A and 2C as virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. *Journal of Virology, 76*, 8551–8559.

Evans, M. S., Clarke, T. F., & Clark, P. L. (2005). Conformations of co-translational folding intermediates. *Protein and Peptide Letters, 12*, 189–195.

Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U., & Ball, L. A. (2005). *Virus taxonomy, eighth report of the international committee on taxonomy of viruses*. New York, NY: Elsevier Academic Press.

Feigelstock, D., Thompson, P., Mattoo, P., Zhang, Y., & Kaplan, G. G. (1998). The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. *Journal of Virology, 72*, 6621–6628.

Franco, E., & Vitiello, G. (2003). Vaccination strategies against hepatitis A in southern Europe. *Vaccine, 21*, 696–697.

Fujiwara, K., Yokosuka, O., Ehata, T., Saisho, H., Saotome, N., Suzuki, K., et al. (2002). Association between severity of type A hepatitis and nucleotide variations in the 5′ non-translated region of hepatitis A virus RNA: Strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut, 51*, 82–88.

Fujiwara, K., Yokosuka, O., Fukai, K., Imazeki, F., Saisho, H., & Omata, M. (2001). Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. *Journal of Hepatology, 35*, 112–119.

Fujiwara, K., Yokosuka, O., Imazeki, F., Saisho, H., Saotome, N., Suzuki, K., et al. (2003). Analysis of the genotype-determining region of hepatitis A viral RNA in relation to disease severities. *Hepatology Research, 25*, 124–134.

Gabrieli, R., Sánchez, G., Macaluso, A., Cenko, F., Bino, S., Palombi, L., et al. (2004). Hepatitis in Albanian children: Molecular analysis of hepatitis A virus isolates. *Journal of Medical Virology, 72*, 533–537.

Gagneux, P., & Varki, A. (1999). Evolutionary considerations in relating oligosaccharide diversity to biological function. *Glycobiology, 9*, 747–755.

Gavrilin, G., Cherkasova, E. A., Lipskaya, G. Y., Kew, O. M., & Agol, V. I. (2000). Evolution of circulating wild poliovirus and vaccine-derived poliovirus in an immunodeficient patient: A unifying model. *Journal of Virology, 74*, 7381–7390.

Germinario, C., Luigi Lopalco, P., Chicanna, M., & Da Villa, G. (2000). From hepatitis B to hepatitis A and B prevention: The Puglia (Italy) experience. *Vaccine, 18*, 3326.

Glikson, M., Galun, E., Oren, R., Tur-Kaspa, R., & Shouval, D. (1992). Relapsing hepatitis A. Review of 14 cases and literature survey. *Medicine (Baltimore), 71*, 14–23.

Graff, J., Richards, O. C., Swiderek, K. M., Davis, M. T., Rusnak, F., Harmon, S. A., et al. (1999). Hepatitis A virus capsid protein VP1 has a heterogeneous C terminus. *Journal of Virology, 73*, 6015–6023.

Gust, I. D. (1992). A vaccine against hepatitis A—at last. *Medical Journal of Australia, 157*, 345–346.

Halliday, M. L., Kang, L.-Y., Zhou, T.-Z., Hu, M.-D., Pan, Q.-C., Fu, T.-Y., et al. (1991). An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *Journal of Infectious Diseases, 164*, 852–859.

Hollinger, F. B., & Emerson, S. U. (2007). Hepatitis A virus. In D. M. Knipe & P. M. Howley (Eds.), *Fields virology* (pp. 911–947). Philadelphia: Lippincott Williams and Wilkins.

Jackson, T. A. (2002). Proteins involved in the function of picornavirus internal ribosome entry sites. In B. L. W. E. Semler (Ed.), *Molecular biology of picornaviruses* (pp. 171–186). Washington, DC: ASM Press.

Kuechler, E., Seipelt, J., Liebig, H.-D., & Sommergruber, W. (2002). Picornavirus proteinase-mediated shutoff of host cell translation: Direct cleavage of a cellular initiation factor. In B. L. W. E. Semler (Ed.), *Molecular biology of picornaviruses* (pp. 301–312). Washington, DC: ASM Press.

Leong, L. E. C., Cornell, C. T., & Semler, B. L. (2002). Processing determinants and functions of cleavage products of picornavirus. In B. L. W. E. Semler (Ed.), *Molecular biology of picornaviruses* (pp. 187–198). Washington, DC: ASM Press.

Leoni, E., Bevini, C., Degli Esposti, S., & Graziano, A. (1998). An outbreak of intrafamiliar hepatitis A associated with clam consumption: Epidemic transmission to a school community. *European Journal of Epidemiology, 14*, 187–192.

Lu, L., Ching, K. Z., de Paula, V. S., Nakano, T., Siegl, G., Weitz, M., et al. (2004). Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). *Journal of General Virology, 85*, 2943–2952.

Mackowiak, P. A., Caraway, C. T., & Portnoy, B. L. (1976). Oyster-associated hepatitis: Lessons from the Louisiana experience. *American Journal of Epidemiology, 103*, 181–191.

Martin, A., Bénichou, D., Chao, S. F., Cohen, L., & Lemon, S. M. (1999). Maturation of the hepatitis A virus capsid protein VP1 is not dependent on processing by the 3Cpro proteinase. *Journal of Virology, 73*, 6220–6227.

Martin, A., & Lemon, S. M. (2006). Hepatitis A virus: From discovery to vaccines. *Hepatology, 43*, S164–S172.

Mele, A., Rastelli, M. G., Gill, O. N., di Bisceglie, D., Rosmini, F., Pardelli, G., et al. (1989). Recurrent epidemic hepatitis A associated with consumption of raw shellfish, probably controlled through public health measures. *American Journal of Epidemiology, 130*, 540–546.

Nainan, O. V., Xia, G. L., Vaughan, G., & Margolis, H. S. (2006). Diagnosis of hepatitis A virus infection: A molecular approach. *Clinical Microbiology Reviews, 19*, 63–79.

Noble, R. C., Kane, E. M., Reeves, S. A., & Roeckle, I. (1984). Posttransfusional hepatitis A in a neonatal intensive care unit. *The Journal of the American Medical Association, 252*, 2711–2715.

Pintó, R. M., Alegre, D., Domínguez, A., El Senousy, W. M., Sánchez, G., Villena, C., et al. (2007a). Hepatitis A virus in urban sewage from two Mediterranean countries. *Epidemiology and Infection, 135*, 270–273.

Pintó, R. M., Aragonès, L., Costafreda, M. I., Ribes, E., & Bosch, A. (2007b). Codon usage and replicative strategies of hepatitis A virus. *Virus Research, 127*, 158–163.

Pintó, R. M., & Bosch, A. (2008). Rethinking virus detection in food. In M. Koopmans, D. O. Cliver, & A. Bosch (Eds.), *Foodborne viruses: Progress and challenges* (pp. 171–188). Washington, DC, USA: ASM Press.

Pintó, R. M., Costafreda, M. I., & Bosch, A. (2009). Risk assessment in shellfish-borne outbreaks of hepatitis A. *Applied and Environmental Microbiology, 75*, 7350–7355.

Previsani, N., Lavanchy, D., & Siegl, G. (2004). Hepatitis A. In I. K. Mushahwar (Ed.), *Viral hepatitis molecular biology diagnosis epidemiology and control* (pp. 1–30). California: Elsevier.

Probst, C., Jecht, M., & Gauss-Muller, V. (1999). Intrinsic signals for the assembly of hepatitis A virus particles. Role of structural proteins VP4 and 2A. *Journal of Biological Chemistry, 274*, 4527–4531.

Reid, T. M. S., & Robinson, H. G. (1987). Frozen raspberries and hepatitis A. *Epidemiology and Infection, 98*, 109–112.

Robertson, B. H., Jansen, R. W., Khanna, B., Totsuka, A., Nainan, O. V., Siegl, G., et al. (1992). Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *Journal of General Virology, 73*, 1365–1377.

Robinson, M., Lilley, R., Little, S., Emtage, J. S., Yarranton, G., Stephens, P., et al. (1984). Codon usage can affect efficiency of translation of genes in *Escherichia coli*. *Nucleic Acids Research, 12*, 6663–6671.

Roos, B. (1956). Hepatitis epidemic transmitted by oysters. *Svenska Läkartidningen, 53*, 989–1003.

Rosemblum, L. S., Mirkin, I. R., Allen, D. T., Safford, S., & Hadler, S. C. (1990). A multistate outbreak of hepatitis A traced to commercially distributed lettuce. *American Journal of Public Health, 80*, 1075–1080.

Sánchez, G., Aragonès, L., Costafreda, M. I., Ribes, E., Bosch, A., & Pintó, R. M. (2004). Capsid region involved in hepatitis a virus binding to glycophorin A of the erythrocyte membrane. *Journal of Virology, 78*, 9807–9813.

Sánchez, G., Bosch, A., Gomez-Mariano, G., Domingo, E., & Pintó, R. M. (2003a). Evidence for quasispecies distributions in the human hepatitis A virus genome. *Virology, 315*, 34–42.

Sánchez, G., Bosch, A., & Pintó, R. M. (2003b). Genome variability and capsid structural constraints of hepatitis A virus. *Journal of Virology, 77*, 452–459.

Sánchez, G., Pintó, R. M., Vanaclocha, H., & Bosch, A. (2002). Molecular characterization of hepatitis a virus isolates from a transcontinental shellfish-borne outbreak. *Journal of Clinical Microbiology, 40*, 4148–4155.

Schultheiss, T., Emerson, S. U., Purcell, R. H., & Gauss-Muller, V. (1995). Polyprotein processing in echovirus 22: A first assessment. *Biochemical and Biophysical Research Communications, 217*, 1120–1127.

Schultheiss, T., Kusov, Y. Y., & Gauss-Muller, V. (1994). Proteinase 3C of hepatitis A virus (HAV) cleaves the HAV polyprotein P2–P3 at all sites including VP1/2A and 2A/2B. *Virology, 198*, 275–281.

Sheretz, R. J., Russell, B. A., & Reunman, P. D. (2005). Transmission of hepatitis A by transfusion of blood products. *Archives of Internal Medicine, 1441*, 1579–1580.

Silberstein, E., Xing, L., van de, B. W., Lu, J., Cheng, H., & Kaplan, G. G. (2003). Alteration of hepatitis A virus (HAV) particles by a soluble form of HAV cellular receptor 1 containing the immunoglobin-and mucin-like regions. *Journal of Virology, 77*, 8765–8774.

Sjogren, M. H., Tanno, H., Fay, O., Sileoni, S., Cohen, B. D., Burke, D. S., et al. (1987). Hepatitis A virus in stool during clinical relapse. *Annals of Internal Medicine, 106*, 221–226.

Sorensen, M. A., Kurland, C. G., & Pedersen, S. (1989). Codon usage determines translation rate in *Escherichia coli*. *Journal of Molecular Biology l, 207*, 365–377.

Stene-Johansen, K., Jenum, P. A., Hoel, T., Blystad, H., Sunde, H., & Skaug, K. (2002). An outbreak of hepatitis A among homosexuals linked to a family outbreak. *Epidemiology and Infection, 129*, 113–117.

Stene-Johansen, K., Tjon, G., Schreier, E., Bremer, V., Bruisten, S., Ngui, S. L., et al. (2007). Molecular epidemiological studies show that hepatitis A virus is endemic among active homosexual men in Europe. *Journal of Medical Virology, 79*, 356–365.

Stroffolini, T., Biagini, W., Lorenzoni, L., Palazzesi, G. P., Divizia, M., & Frongillo, R. (1990). An outbreak of hepatitis A in young adults in central Italy. *European Journal of Epidemiology, 6*, 156–159.

Tallo, T., Norder, H., Tefanova, V., Ott, K., Ustina, V., Prukk, T., et al. (2003). Sequential changes in hepatitis A virus genotype distribution in Estonia during 1994 to 2001. *Journal of Medical Virology, 70*, 187–193.

Tanaka, J. (2000). Hepatitis A shifting epidemiology in Latin America. *Vaccine, 18*, S57–S60.

Teppakdee, A., Tangwitoon, A., Khemasuwan, D., Tangdhanakanond, K., Suramaethakul, N., Sriratanaban, J., et al. (2002). Cost-benefit analysis of hepatitis a vaccination in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health, 33*, 118–127.

Tortajada, C., de Olalla, P. G., Pintó, R. M., Bosch, A., & Cayla, J. (2009). Outbreak of hepatitis A among men who have sex with men in Barcelona, Spain, September 2008–March 2009. *Eurosurveillance* 14(15): pii: 19175.

Van Damme, P., & Van Herck, K. (2005). Effect of hepatitis A vaccination programs. *JAMA-Journal of the American Medical Association, 294*, 246–248.

Wasley, A., Samandari, T., & Bell, B. P. (2005). Incidence of hepatitis A in the United States in the era of vaccination. *Journal of the American Medical Association, 294*, 194–201.

Weitz, M., Baroudy, B. M., Maloy, W. L., Ticehurst, J. R., & Purcell, R. H. (1986). Detection of a genome-linked protein (VPg) of hepatitis A virus and its comparison with other picornaviral VPgs. *Journal of Virology, 60*, 124–130.

Whetter, L. E., Day, S. P., Elroystein, O., Brown, E. A., & Lemon, S. M. (1994). Low efficiency of the 5′ nontranslated region of hepatitis A virus RNA in directing cap-independent translation in permissive monkey kidney cells. *Journal of Virology, 68*, 5253–5263.