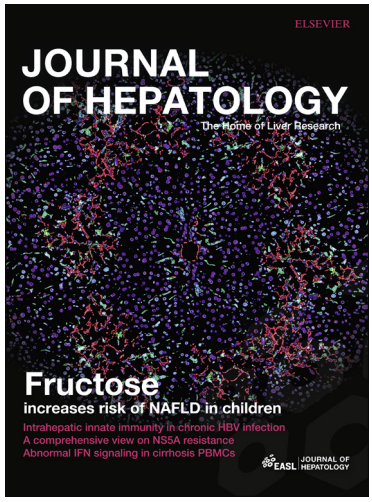
Type A viral hepatitis : a summary and update on the molecular virology, epidemiology, pathogenesis and prevention



Seminar

Type A viral hepatitis: A summary and update on the molecular virology, epidemiology, pathogenesis and prevention

Stanley M. Lemon, Jördis J. Ott, Pierre Van Damme, Daniel Shouval

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Type A viral hepatitis: A summary and update on the molecular virology, epidemiology, pathogenesis and prevention.

Authors in alphabetical order; equal contribution:

Stanley M. Lemon[1,**], Jördis J. Ott[2,3,*,**], Pierre Van Damme[4,**], Daniel Shouval[5,**]

[1] Lineberger Comprehensive Cancer Center, and the Departments of Medicine and Microbiology & Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7292, USA; smlemon@med.unc.edu

[2] Department of Epidemiology, Helmholtz Centre for Infection Research, Braunschweig, Germany

[3] Hannover Medical School, Hannover, Germany

[4] Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute, Antwerp University, Antwerp, Belgium

[5] Liver Unit, Institute for Gastroenterology and Hepatology, Hadassah-Hebrew University Hospital, P.O.Box 12000, Jerusalem 91120, Israel;

** Equal contribution.

*** Corresponding author:**

Dr. Jördis J. Ott, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124 Braunschweig, Germany. Email: Joerdis.Ott@helmholtz-hzi.de; Phone: +49-531 6181 3115.

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Abbreviations: Advisory Committee on Immunization Practices (ACIP), alanine aminotransferase (ALT), hepatitis A virus (HAV), anti-HAV immunoglobulin G (IgG) antibodies (anti-HAV IgG), Disability Adjusted Life Years (DALY), Hepatitis B Virus

(HBV), extracellular vesicle (EV), quasi-enveloped HAV (eHAV), *cis*-acting replication element (*cre*), enzyme-linked immunosorbent assay (ELISA), endosomal sorting complexes required for transport (ESCRT), geometric mean titer concentration (GMC), immune globulin (IG), genome equivalents (GE), intramuscular injection (i.m.), internal ribosome entry site (IRES), intraluminal vesicle (ILV), mitochondrial antiviral-signaling protein (MAVS), men who have sex with men (MSM), multivesicular body (MVB), National Health and Nutrition Examination Survey (NHANES), open reading frame (ORF), universal mass vaccination (UMV), untranslated RNA (UTR), post-exposure prophylaxis (PEP), plasmacytoid dendritic cell (pDC), subcutaneous injection (s.c.), World Health Organization (WHO), European Centre for Disease Prevention and Control (ECDC), Centers for Disease Control and Prevention (CDC), European Union (EU), European Economic Area (EEA), Global burden of disease (GBD), United States of America (US).

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Key points:

- HAV is a small positive-strand RNA virus that is shed in feces as naked non-enveloped virions but circulates in blood cloaked in host membranes.

- HAV infection causes an acute necro-inflammatory process in the liver that resolves spontaneously without chronic sequelae.

- HAV replicates in hepatocytes without a cytopathic effect; liver injury is caused by innate and adaptive immune responses to HAV.

- The clinical outcome of HAV is strongly age-related with infection in young children often asymptomatic and older age groups at risk for more severe disease.

- Changes in global HAV-epidemiology predict a potential for outbreaks and shifts in vulnerable populations.

- HAV infection is a preventable disease using inactivated or live attenuated vaccines for pre-exposure prophylaxis

- The use of HAV immune serum globulin (IG) for pre and post-exposure prophylaxis is declining

- New methods to distinguish between HAV immunity from natural infection and HAV immunity from vaccination are needed and can enhance prevention policies

1. Introduction

Hepatitis A virus (HAV) infection is an ancient disease and likely to have afflicted mankind since humans first began to live in groups large enough to sustain transmission of the causative agent, HAV. In reviewing what was known as 'catarrhal jaundice' in 1912, Cockayne noted descriptions of epidemic jaundice extending back to antiquity[1]. The infectious nature of the disease was proven several decades later in deliberate human transmission studies[2]. Such experiments led to a clear distinction between hepatitis A ('infectious hepatitis') and hepatitis B ('homologous serum jaundice') and recognition of the lack of cross immunity between these two forms of transmissible hepatitis as early as in 1945[3]. However, the responsible virus was not identified until almost 30 years later, when small, round viral particles were discovered by immune electron microscopy in the feces of an experimentally-infected human subject by Feinstone et al. in 1973[4]. This review provides an up-to-date in in-depth overview of HAV and the acute inflammatory hepatic infection it causes in

humans, including recently recognized aspects of its molecular virology, evolution, natural history, pathogenesis, epidemiology and prevention.

2. The molecular virology of HAV

Genome organization and virion structure. The molecular cloning of the RNA genome of HAV in the early 1980s revealed its organization to be similar to the genomes of poliovirus and other viruses classified with the family *Picornaviridae*[5,6]. HAV is now classified taxonomically within a unique picornaviral genus, the genus *Hepatovirus,* which includes only human HAV and other closely related mammalian viruses[7]. Its single-stranded, positive-sense RNA genome is approximately 7.5 kb in length, with a lengthy 5' untranslated RNA (UTR) segment covalently linked at its 5' terminus to a small virally-encoded protein, VPg (or 3B) (Fig. 1). The RNA contains a single open reading frame (ORF) that encodes a giant polyprotein, the translation of which initiates under control of a highly structured internal ribosome entry site (IRES) within the 5'UTR. Upstream of the IRES, there is a 5' terminal hairpin structure followed by two putative RNA pseudoknots and a conserved poly-U/UC tract, the latter of which is expendable for replication and virulence in primates and thus of unknown function[8-10]. Within the ORF, there is a complex internal stem-loop which functions as an essential *cis*-acting RNA replication element (*cre*)[11] (Fig. 1). The genome terminates downstream of the ORF in a short 3'UTR segment ending in a 3' poly(A) tail. As with other positive-strand RNA viruses, synthetic genome-length RNA is infectious and generates virus when transfected into permissive cell cultures[12].

There are two forms of infectious virus (Fig. 1). Naked, non-enveloped HAV virions shed in feces by infected persons are small, 27nm diameter icosahedral protein capsids within which the RNA genome is packaged[13]. The antigenic structure of the capsid is highly conserved, and all human hepatoviruses form a single serotype[14]. This antigenic conservation extends to novel HAV-like viruses identified recently in bat livers that bind some monoclonal antibodies targeting human HAV[15]. Quasi-enveloped virions (eHAV) that are secreted non-lytically from infected cells represent a second form of infectious virus[16]. These virions are found in the blood and in supernatant fluids of infected cell cultures, and are comprised of RNA-containing capsids enclosed within membranous vesicles that have no virally-encoded proteins on their surface. They represent an unusual feature of HAV that

has been recognized only recently, and that figures prominently in the pathogenesis of the infection.

Polyprotein processing and RNA genome amplification. The HAV replication cycle commences with translation of the polyprotein which occurs in a 5' cap-independent manner under control of the IRES in the 5'UTR. The polyprotein is processed co-translationally into 10 mature viral proteins that are named according to picornaviral conventions. These include, from the N-terminus, 4 structural proteins that form the capsid, VP4, VP2, VP3 and VP1pX (numbered according to molecular mass), and 6 nonstructural proteins that are essential for amplification of the RNA genome: 2B, 2C, 3A, 3B (the genome-linked protein, VPg alluded to above), $3C^{pro}$ (a cysteine protease), and $3D^{pol}$ (RNA-dependent RNA polymerase) (Fig. 1). With the exception of the cleavage between VP4-VP2 and a late cleavage within VP1pX found in naked HAV particles, all polyprotein processing events are mediated by $3C^{pro}$, or its precursors (e.g., 3ABC)[17]. $3C^{pro}$ is the only proteinase expressed by the virus.

The mechanisms underlying synthesis of HAV RNA have not been intensively investigated, but are thought to be similar to those of other, well-studied picornaviruses[17,18]. RNA replication proceeds slowly, and like all positive-strand RNA viruses, within the cytoplasm and in close association with membranes. Over-expression of 2BC or 2C leads to extensive rearrangements of intracellular membranes derived from the endoplasmic reticulum[19], likely reflecting a role for these nonstructural proteins in establishing a membranous microenvironment within which RNA synthesis can proceed. RNA synthesis probably occurs within a tubular-vesicular network that has been observed by electron microscopy in close proximity to the rough endoplasmic reticulum[20]. The (+)-strand RNA is transcribed to a (-)-strand intermediate, resulting in a duplex RNA molecule that then serves as template for the production of multiple new (+)-strand RNA progeny in reactions catalyzed by $3D^{pol}$. Studies with poliovirus suggest RNA synthesis is protein-primed, with a uridylated form of VPg (VPg-pUpU) produced in a reaction templated by the *cre* serving as the primer[11,21]. Replication proceeds in a non-conservative fashion, with new (+)-strands greatly outnumbering new (-)-strand RNA. Recent studies with RNA replicons implicate host cell adenosine-triphosphate-binding cassette (ABC) transporters and FK506-binding proteins in the replication cycle[22]. It is likely that additional host cell proteins, such as PCBP2[23], are also important. Much of this will

sound familiar to anyone who has followed recent studies of the replication of hepatitis C virus (HCV), also a positive-strand RNA virus. Important to the pathogenesis of hepatitis A, replication is non-cytopathic, with newly assembled virions exiting the cell in a non-lytic fashion. However, highly passaged, cell culture-adapted variants may induce apoptotic cell death[20,24].

Capsid assembly, quasi-envelopment, and cellular egress. Three capsid precursor proteins, VP4-2 (also known as VP0), VP3 and VP1pX, fold into 'protomers' which then assemble as pentamer subunits with antigenic activity similar to the native capsid[25]. Further assembly of 12 of these pentamer subunits into complete capsids is dependent upon VP4 sequence[26]. The sequence of events leading to RNA packaging is not well understood, but cleavage at the VP4-2 junction does not proceed until the immature capsid has packaged RNA. Recent X-ray crystallography studies show that the mature HAV capsid is remarkably different from that of other mammalian picornaviruses, such as poliovirus or foot-and-mouth disease virus[13]. The VP2 protein assumes a very different fold, with its N terminus extending across the 2-fold axis of symmetry to the adjacent protomer, a feature seen only in primitive picorna-like viruses of insects. This may contribute to the high resistance of HAV to heat and acid inactivation[27,28], and has implications for the evolution of HAV as a truly ancient virus. Consistent with this, 12 distinct hepatovirus species have been identified recently in tissues and droppings from bats, shrews, hedgehogs, rodents, and even seals [15,29,30]. Phylogenetic analyses indicate these viruses have jumped between host species in the distant past, and ancestral reconstructions suggest HAV is likely to have originated ultimately in a small insectivorous mammal[15].

Quasi-enveloped eHAV virions are released from infected cells non-lytically as small extracellular vesicles (EVs), the membranes of which completely envelope and protect the capsid from neutralizing antibodies[16,31]. These membrane-wrapped particles possess a specific infectivity similar to naked HAV virions, are ~50-110nm in diameter, and have a buoyant density of ~1.100 gm/cm^3 in iodixanol[16]. They share a several features in common with conventional enveloped viruses, but express no virally encoded glycoproteins on their surface[31]. They are the only form of virus found in sera from infected humans or experimentally-infected chimpanzees[16]. In terms of

their size and density, these eHAV vesicles resemble 'exosomes', small EVs that function in intercellular communications[32].

Exosomes and eHAV share a common mechanism of biogenesis involving secretion through the multivesicular body (MVB) pathway[16]. Viral capsids are recruited from the site of RNA replication and packaging to late endosomes, into which they bud to form MVBs in a process mediated by endosomal sorting complexes required for transport (ESCRT) (Fig. 2). HAV capsids are recruited as cargo for export via the MVB pathway through interactions with ALIX, an accessory ESCRT-III protein[33]. Two tandem YPX_3L motifs, separated by 28 residues in the VP2 capsid protein, function as 'late domains' that mediate interactions of the capsid with ALIX, a protein that is also involved in the budding of multiple conventional enveloped viruses, including human immunodeficiency virus[34,35]. siRNA knockdown of ALIX and several other late ESCRT complex-associated proteins, including VPS4B, inhibits eHAV release, and recent proteomics studies show both ALIX and VPS4 are physically present in extracellular eHAV[16,36]. MVBs with intraluminal vesicles (ILVs) containing HAV capsids then move to the plasma membrane, where fusion of the limiting membrane of the MVB with the plasma membrane results in extracellular release of the ILVs as eHAV (Fig. 2).

Consistent with the presence of quasi-enveloped eHAV in blood during acute hepatitis A[16], eHAV is released from both basolateral (hepatic sinusoid) membranes as well as apical (biliary) membranes of polarized HepG2-N6 cells[37]. Virus released across the apical membrane of hepatocytes is stripped of its membranes by the detergent action of bile salts during passage through the biliary track, and is thus shed in feces as naked virions[37,38]. Interestingly, hepatitis E virus (HEV), yet another hepatotropic positive-strand RNA virus that is phylogenetically unrelated to HAV, appears to have evolved a similar mechanism to exit infected hepatocytes nonlytically[31].

Cellular entry of HAV and eHAV. A detailed understanding of how eHAV enters cells is lacking, but this appears to occur via an endocytic pathway, with slow removal of the membranes rendering the capsid subject to neutralization by antibody in an endosomal compartment over a period of 6-8 hrs[16]. The lysosomal poison, chloroquine, strongly inhibits eHAV entry[16], and it seems likely that the eHAV membrane is degraded by lysosomal enzymes. Removal of the membrane allows the

capsid to access a specific cellular receptor. In contrast, naked HAV uncoats rapidly, does not require endosomal acidification, and is not subject to post-endocytic neutralization[16]. It is likely to interact with the same receptor, but in an early endosome. TIM-1 (HAVCR1) has been suggested to function as an HAV receptor[39,40], and it cycles dynamically between the plasma membrane and lysosomes in a process involving clathrin-mediated endocytosis[41]. However, the X-ray structure of the virus capsid shows no 'canyon' surrounding the 5-fold axis of symmetry into which a receptor might fit (as is the case in the enteroviruses)[13]. Moreover, recent studies in cell culture and in mice with defects in innate immunity have shown that TIM1, long believed to be the HAV receptor, is in fact not an essential HAV entry factor[42]. Additional studies are needed to define entry mechanisms for both eHAV and naked HAV virions.

3. The pathogenesis of hepatitis A

Animal models of hepatitis A. In addition to humans, chimpanzees (*Pan troglodytes*), and several small nonhuman primates are susceptible to HAV[43-46]. Infection has also been achieved recently by intravenous inoculation of mice genetically deficient either for IFNAR1, a key component of the type I interferon receptor, or for signaling molecules (MAVS or IRF3/IRF7) involved in the induction of interferon responses to viral infection[38]. Infections in these animal species have provided valuable models for studies of HAV pathogenesis and/or vaccine development.

Although infection is typically acquired by ingestion of the virus, HAV is thought to replicate primarily if not exclusively within hepatocytes. The infecting HAV particle presumably arrives at the basolateral membrane of the hepatocyte, within the space of Disse, via the portal circulation. A primary site of replication within epithelial cells lining crypts of the small intestine has been suggested[47], but this is not well documented. An alternative hypothesis, first proposed for poliovirus[48], would be that virus is directly transported into the submucosal space and thence the blood via transcytosis across specialized "M" cells overlying intestinal Peyer's patches.

Nonhuman primates can be infected experimentally either by enteric or intravenous inoculation, with infection by either route leading to extensive shedding

of virus in the feces, much or all of which is derived from the liver and reaches the intestines via bile. In infected *Ifnar1*$^{-/-}$ and *Mavs*$^{-/-}$ mice, there is little if any viral RNA in intestinal tissues despite abundant fecal shedding of virus[38]. Unlike the quasi-enveloped virus circulating in blood, virus shed in feces is entirely naked and non-enveloped, having lost its membrane during passage through the biliary system[37]. Fecal shedding is maximal during the latter part of the typical 3-4 week incubation period, just prior to the onset of liver injury[47,49,50]. This diminishes rapidly with the onset of symptoms and biochemical evidence of liver injury (alanine aminotransferase [ALT] elevation), which occurs concomitantly with the appearance of antibodies. However, viral RNA can be found by sensitive RT-PCR assays within feces for several weeks, and within the liver for many months after acute infection[50]. Whether this reflects the presence of infectious virus is uncertain, as infectivity is masked by the presence of potent neutralizing antibodies. Viremia is present throughout much of the incubation period and into the acute illness, but like fecal shedding is reduced in magnitude with the onset of hepatitis and the appearance of anti-HAV antibodies[47,49].

Liver injury and the host immune response to HAV. Acute infection with HAV evokes only minimal intrahepatic type I IFN responses in chimpanzees (Fig. 3)[50]. This may be due, at least in part, to the ability of 3C^{pro} precursors, 3ABC and 3CD, to proteolytically cleave and functionally eliminate MAVS and TRIF, key signaling molecules involved in the induction of interferon responses[51,52]. Interestingly, these same signaling proteins are targeted by the hepatitis C protease, NS3/4A [53,54]. The mature HAV protease, 3C^{pro}, also cleaves NEMO, a bridging adaptor required for NF-κB activation and IFN-β expression [55]. Plasmacytoid dendritic cells (pDCs), which typically produce large amounts of type 1 interferon in response to virus infections, respond robustly when placed in co-culture with HAV-infected cells or exposed to eHAV[56]. These cells can be detected within hepatic sinusoids by the end of the first week of infection in chimpanzees, but subsequently disappear and are not present at the peak of virus replication [56].

Since maximal fecal shedding generally precedes the onset of disease, HAV replication by itself does not cause hepatocellular injury. Early studies suggested liver injury might result from a robust T cell response, as IFN-γ-producing, virus-specific, cytotoxic CD8+ T cells have been identified in peripheral blood and liver of infected

humans[57,58]. Consistent with this, a strong multi-specific T cell response was observed by intracellular cytokine staining *ex vivo* in peripheral blood mononuclear cells isolated from acutely infected humans, with specific epitopes identified by tetramer staining[59]. However, studies in experimentally-infected chimpanzees suggest a more nuanced T cell response to the virus and cast doubt on the role of cytotoxic T cells as a cause of liver injury. CD4+ helper T cells predominated over CD8+ T cell responses in a careful study of cells isolated directly from chimpanzee liver and blood[60]. Virus-specific CD8+ T cells were either not detected, or failed to display significant effector functions until after viremia and liver injury had peaked. A multi-functional CD4+ helper T cell response correlated well with resolution of the infection and elimination of virus from the liver [60].

Choi and colleagues[61] reported that the magnitude of the circulating regulatory T cell (Treg) pool was reduced in Korean patients with acute hepatitis A due to Fas-mediated apoptosis, and that this was associated with greater ALT elevations. However, the absence of a correlation between Treg frequency and circulating HAV-specific CD8+ T cells suggests the latter are probably not responsible for liver injury. Interactions between the HAV capsid and TIM-1 may modulate Treg function [62], and possibly also the cytotoxic activity of NKT cells [63]. In addition, recent studies in *Ifnar1*$^{-/-}$ mice show that acute ALT elevation and the hepatocellular apoptosis that typifies the pathology of acute hepatitis A can be mediated entirely by innate immune activation of MAVS and IRF3/IRF7 in the absence of CD4+ or CD8+ T cells, or for that matter NK cells[38]. Thus, multiple immune mechanisms may contribute to acute liver injury in humans.

Antibody responses to HAV. The specific diagnosis of acute hepatitis A rests upon demonstration of serum IgM antibody to HAV (or seroconversion to anti-HAV positive status) in an individual with a compatible clinical picture.[64] Antibody responses are similar in human and in experimentally-infected nonhuman primates. In general, the B cell response to HAV infection is delayed[50], possibly because HAV antigens are sequestered within non-lytically infected cells, and released only in host membrane-cloaked quasi-enveloped virions[16]. However, plasmablasts secreting IgM with multiple specificities are present by the onset of symptoms [65], and transcripts of genes involved in B cell development are among the most robustly upregulated in microarray studies of intrahepatic transcriptional responses in chimpanzees[50]. These

include transcripts for multiple immunoglobulin genes, and for CXCLX13, a chemokine that recruits B cells to the liver. A neutralizing IgG response to the virus rapidly becomes dominant and provides life-long protection against symptomatic reinfection[66]. These neutralizing antibodies recognize a small number of closely-positioned epitopes in the highly conserved VP1, VP2, and VP3 capsid proteins [13,67]. Antibody directed against the capsid may comprise as much as 10% of all circulating IgG antibodies by several months after infection.

Passively transferred anti-HAV antibodies (in the form of pooled human immune globulin (IG)) or post-exposure immunization with inactivated HAV vaccine can prevent liver disease if administered within two weeks of exposure to the virus [68]. While very well documented, this is surprising inasmuch as the eHAV circulating in blood at this point in the infection is cloaked in membranes and resistant to antibody-mediated neutralization in conventional infectious focus reduction assays[16]. The positive effects of antibody at this relatively late stage of infection, when virus replication is well-established within the liver[50], likely results from post-endocytic neutralization within late endosomes or lysosomes following removal of the membrane from the capsid[16]. This would reduce viral load within the liver, which seems to be a key determinant of liver injury.

Neutralizing antibodies may similarly contribute to resolution of the acute infection, although the lack of reports of persistent HAV infections in IgG-deficient persons would argue against a key role. The expansion of multi-functional virus-specific CD4+ T cells correlates well with declines in intrahepatic viral RNA in experimentally-infected chimpanzees[60], and it seems likely that these cells may be the primary means of control of the infection by producing antiviral cytokines[69,70]. HAV is also quite sensitive to interferons and despite the rather paltry interferon response observed in chimpanzees, interferon-stimulated genes (ISGs), including ISG15, IFITs, and others, may also play an important role[38,50]. Among the factors influencing the clinical course of HAV infection in humans, virulence has been associated with particular viral nucleotide sequence variations at the 5' non-translated region of HAV. This however, requires further study[71,72].

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4. The epidemiology of HAV

An estimated 1,5 million people are infected annually with HAV[73]. This figure is most probably an underestimate due to the asymptomatic presentation of hepatitis A and the limitations regarding epidemiologic information on HAV. Infection with HAV occurs via person to person contact and is mainly acquired through fecal-oral transmission, as a result of exposure to contaminated water and food. HAV is relatively resistant to freezing, to low pH and to inactivation by moderate heating as well as chemical and physical agents. Thus, it has the ability to survive on environmental surfaces, human skin, food items and sewage[74].

The population incidence of HAV infection is related to socio-economic conditions including density of housing, sanitation, quality of water, and income. Overall improvements of such conditions worldwide are leading to a shift of susceptibility to infection from early age to young and even older adults.

The paradox of HAV epidemiology. Traditionally, the endemicity of HAV is classified into low, intermediate, and high levels. These groupings are made based on the prevalence of anti-HAV IgG in human serum and reflect seropositivities of <15%, 15-50%, and > 50%, respectively in the studied population.[75] However, the interpretation of endemicity levels and of corresponding HAV-risk maps differs from other viruses and has led to misinterpretations. The “paradox of hepatitis A risk” has been described in the context of global mapping efforts[76] and is expressed by the fact that a high sero-prevalence of anti-HAV IgG antibodies reflects high endemicity, meaning high levels of population immunity. Importantly, infections in very young children are typically asymptomatic or at least not recognized clinically as hepatitis, whereas frank hepatitis typifies infection in adults as described below. High endemicity is associated with infection at an early age, and thus paralleled by a low disease burden and a low population infection susceptibility, which reflects the proportion of vulnerable adults that escaped exposure to HAV in early childhood. The endemicity can be further staged according to age, thus a highly HAV endemic region like Sub- Saharan Africa is characterized by over 90% of individuals being anti-HAV IgG positive at the age of 10 years[73].

As a core reference for global HAV assessment, Jacobsen & Wiersma (2010)[77] estimated the age-specific anti- HAV seroprevalence for 21 world regions, based on published data (Fig. 4). More recently, the *GBD 2013 Mortality and Causes of Death Collaborators* [78] published data indicating that HAV caused 14,900 deaths in 2013

with an age-standardized death rate of 0.2 per 100,000. This was accompanied by median reductions of around 40% compared to 1990. The *Foodborne Disease Burden Epidemiology Reference group*, on the other hand, estimated the contribution of HAV specifically to foodborne diseases and reported that HAV was responsible for 14 million foodborne illnesses, 27,731 foodborne deaths, and around 1.4 DALYs worldwide in 2010, with great variations by region [79]. These global evaluations are based on different original data, disease categories and they apply varying methods and life tables. For example, Havelaar et al. (2015) [79] used incidence-based DALYs whereas models applied by the *GBD collaborators* [78] utilized sero-prevalence of antibodies or antigens for HAV.

Researchers and health policy makers uniformly allude to the sparsity, low quality, and lack of comparability of available HAV data. Ideally, such data should be derived from sufficiently powered serological surveys that measure age-specific seroprevalence of anti-HAV IgG [73], *i.e.* immunity to HAV in the general population . However, only a limited number of publications are available providing (national) sero- epidemiological HAV information based on prospectively collected sera in the general population with adequate sample size. Specifically, more recent data from low and middle income countries are limited [77], but even from numerous European countries, no sero-epidemiological data are available at all [80,81].

Other methods have been used to describe the epidemiology of HAV in some countries, such as the analyses of serum stored at hospital laboratories for diagnostic purposes (e.g. [82]). Yet, in countries that report HAV notifications, notifiable disease records or surveillance data on new HAV cases remain the main and only available source for epidemiologic HAV information. These are crucial for the detection of outbreaks and infection dynamics but have certain limitations, which restrict their validity and sufficiency for providing details on population HAV susceptibility and immunity. First, notification records only capture symptomatic HAV infections which are to be, depending on country, additionally serologically confirmed [83,84]. Given the strong interrelation between the probability of symptom appearance and age of infection, this can introduce reporting bias and results in underestimation of reported HAV cases [85]. Second, information on HAV infection outcome and severity is not reported routinely and even within EU/EEA countries, varying case definitions are applied for surveillance [85,86] that challenge comparability and explanatory power of the records.

The lack and limitations of existing epidemiologic HAV data is particular serious as the infection has undergone pronounced epidemiologic changes over the last few decades and in most countries. Areas of low and decreasing HAV endemicity/ immunity like the US and most European countries, specifically Nordic countries in Europe are characterized by shifts to intermediate or low and very low endemicity, resulting in low levels of virus circulation and relative increases in susceptible individuals[81]. In some regions these changes lead to increases in the incidence of symptomatic infections among persons who were neither vaccinated nor previously infected and who become infected at older ages, when sequelae are often more severe [80,87-90] (Fig. 5). For example, analyses of the US National Health and Nutrition Examination Survey (NHANES) which systematically collects serum samples, demonstrated decreases in HAV immunity among adults aged 50 to 59 years between survey waves in 1988-94 to 1999-2006 [91]. Regarding the oldest age- group, significant reductions in HAV immunity were obvious in subsequent years, too, i.e. from survey waves 1999-2002 to 2009-2012[92]. In line with this, recent data from the National Notifiable Diseases Surveillance System in the US showed an increasing trend in hospitalization among reported HAV cases, paralleled by an increase in mean age of fatal and lethal HAV cases over the last decade [87]. Modeling projections based on data from the Netherlands confirm these findings and conclude that, under a baseline scenario, by 2030 23.5% of all new HAV infections will occur in individuals aged 55 years and older, compared to 5.5% in this age- group in 2000. Thereby, a compensatory effect for the overall modeled and expected decrease in HAV disease burden will become evident [93].

Given these findings, it is likely that the greater proportion of new HAV infections and severe disease outcomes in the older age groups is a continuing trend; mainly resulting from demographic changes and cohort effects, according to which younger ages that were less frequently exposed to HAV are replacing HAV immune cohorts [88,93]. The overall increase in reported HAV cases/incidence in the EU/EEA countries since 2011[85] points to new risks associated with population movements and market globalization as well as to potentially vulnerable population groups like men who have sex with men[94]. Targeting preventive measures not only towards previously defined high-risk groups like travelers and immunocompromised individuals but also considering adults without other risk factors but exposed and vulnerable might be necessary.

New approaches and the need for further HAV research. In contrast to HBV, where presence of anti-HBs in the absence of anti-HBc enables serologic distinction between humoral immune response to previous infection and immunization, no such marker is available yet for hepatitis A. In addition, no assay for serological investigations has been licensed that specifically distinguishes between naturally and vaccine-induced immunity against HAV. Related information has so far been assessed based on vaccine cards or through self-reported vaccination status, assuming that the detection of HAV antibodies in sera of reported unvaccinated individuals results from natural infection. However, both strategies of anamnestic assessment are less reliable: recall bias can affect self- reporting [95] and low sensitivity in reporting can occur due to lost vaccine cards or undocumented vaccines. Furthermore, while self-reporting their immunization status, individuals are often not able to distinguish between previously received active or passive HAV vaccination [88].

These limitations have made it difficult to determine the origin of antibodies in HAV-immune individuals and to determine the current parameters of HAV epidemiology of critical importance to policy makers. A technique that differentiates the source of HAV immunity could complement health intervention planning and address relevant public health topics.

Previous efforts to develop such a test used immunoprecipitation to detect antibodies to nonstructural HAV proteins that are found only in infected, not vaccinated individuals[96]. While the immunoprecipitation assay provided proof-of-principle that such tests are possible, it was insufficiently sensitive to detect all naturally infected persons. Another approach indicates the general possibility to distinguish between naturally infected and vaccinated children, based on the HAV-polypeptide subunit 3C[97]. Differentiation in this study however solely depends on 3C and has so far not been confirmed and reproduced in studies including numerous individuals and other age-groups, i.e. adults. Future efforts along these lines would be useful, as a sufficiently sensitive and specific assay for previous infection (versus immunization with inactivated vaccines) would allow:

a) the assessment of causes and attributions of changes in sero-status and effectiveness of health interventions, e.g. through longitudinal studies that initially

recruited sero-negative individuals. In fact, HAV provides a case example for this as the single dose vaccine administration is increasingly discussed as a sufficiently protective alternative to per- protocol application of two doses [98-101].

b) the identification of target groups for national vaccination strategies, e.g. through serological surveys. In light of aforementioned population developments, this might be important to account for the observed high HAV immunity in groups like immigrant children, where it could be due to a history of maternal HAV infection or vaccination.

c) the evaluation of duration of serological detectable protection from HAV infection, namely following infection or through vaccination, which might be influenced by the acquisition mode of HAV immunity. Available evidence confirms detectable protection persists for up to at least 15 years, and 14 years for immunity induced by live attenuated and inactivated HAV vaccines, respectively [102]. More recent data reveal persistence of antibodies up to 20 years post-vaccination[103]. Based on modelling approaches, a life- long protection is likely but may differ between vaccine- induced and naturally acquired immunity [104,105]. However, data from early studies done in Costa Rica suggest that older individuals infected early in life may become asymptomatically re-infected if living in a household with an infected child[106].

In conclusion, societal and demographic factors challenge current and future HAV prevention and control activities. These need to be grounded in reliable epidemiologic data, obtained from (repeated and updated) serological surveys and studies that are able to illustrate changes in immunity over time and in certain age cohorts. Phylogenetic epidemiologic analysis is also indicated and enabled by the different HAV genotypes and their geographically different distribution. The obtained information can identify and address gaps in current records, for example, concerning HAV immunity levels in mostly asymptomatic children. Further genetic analysis of the virus can facilitate the identification of a common source in an outbreak by linking isolates from disparate geographic sites over a period of time, as exemplified by the recent identification of Imported frozen pomegranate arils as a vehicle for transmission of HAV in the U.S[107].

New analytical tools that distinguish between sources of acquired immunity, i.e. natural infection versus vaccination and that are applicable on a large scale in population-based surveys would add relevant information for preventive intervention planning and can infer existing vaccine coverage data [108,109]. Such tools and laboratory approaches may additionally be valuable for other pathogens; e.g. those for which new preventive vaccines became available (like HEV) or for agents that vary antigenically and where the source of immunity is strongly correlated with susceptibility.

5. Natural history of HAV and its clinical manifestations

Although there is only a single serotype of human HAV, the identification of several different HAV genotypes and sub-genotypes has significantly enhanced the ability to investigate hepatitis A outbreaks and define HAV transmission routes [110]. *There are* several genetically distinct variants of HAV classified into six genotypes I-VI (defined by a 15% nucleotide variation). Genotypes I-III infect mainly humans while genotypes IV –VI are simian derived. Genotype heterogeneity has been evaluated for evaluation of genetic relatedness of HAV strains using a 168 nucleotide fragment by PCR containing the VP1/VP2A junction. Yet, whole genome sequencing is a more accurate tool for identification of viral strains relatedness. Genetic differences between sub-genotypes are defined by nucleotide variation of ~7-7.5%[71,110,111] (Fig. 6).

Different genotypes allows the molecular tracing of HAV outbreaks and became an essential tool in epidemiologic investigations worldwide. [71,110,112]Acute HAV infection causes an acute necro-inflammatory process in the liver that normally resolves spontaneously without chronic sequelae. The incubation period of hepatitis A is usually 14–28 days (up to 50 days). Symptoms of hepatitis A range from mild to severe, and can include fever, malaise, fatigue, loss of appetite, diarrhea, nausea, abdominal discomfort, anorexia, myalgia, arthralgia, headache, dark-coloured urine and jaundice[113].

Atypical extra-hepatic manifestations of acute hepatitis A are not frequent and include rash, pancreatitis, mono-neuritis, Guillain-Barré syndrome, acute kidney injury including glomerulonephritis as well as interstitial nephritis and renal failure following hemolysis, dehydration or liver failure, pneumonitis, myocarditis, pleural or

pericardial effusion, arthritis, haemolysis (especially in patients with glucose-6 phosphate dehydrogenase deficiency)[114] and aplastic anaemia[115]. Some patients suffer from prolonged fatigue, right upper quadrant discomfort, fat intolerance and indigestion, weight loss, emotional instability and prolonged indirect bilirubinaemia and cholestasis[89,115-117]. Finally, it has been claimed that HAV infection may trigger presentation of autoimmune hepatitis type 1 in genetically susceptible individuals.[118 119]

Serological testing (IgM anti-HAV) will establish the diagnosis of acute hepatitis A. IgM, IgG and IgA anti-HAV antibodies appear shortly before or concurrent with the onset of symptoms. Anti-HAV IgM antibodies are detectable in both symptomatic and asymptomatic patients; in symptomatic patients, these antibodies appear within 5–10 days of symptom onset, which correlates with the phase of greatest liver enzyme increases, and persist for about 4 months (range 30–420 days) [75]. IgG antibody appears simultaneously, but comes to dominate the antibody response and then persist for many years, probably for life in most individuals, after infection. With nucleic acid amplification and sequencing techniques, HAV RNA can be detected in feces and body fluids. This is rarely required or done for diagnostic purposes, however.

Overall, five clinical patterns are recognized: 1) asymptomatic HAV infection, often present in children under the age of 5 years; 2) symptomatic HAV infection with the appearance of dark urine and sometimes clay-coloured stools, often accompanied or followed by jaundice; 3) cholestatic hepatitis characterized by pruritus, prolonged elevation of alkaline phosphates, gamma glutamyl transpeptidase, bilirubinemia and weight loss; 4) relapsing hepatitis A infection manifested by reappearance of the clinical, biochemical and virologic markers of acute hepatitis A after initial resolution; 5) fulminant hepatitis, which can resolve spontaneously but often requires life-saving liver transplantation.

Not everyone infected with HAV will present with all of the symptoms described above. The clinical outcome is strongly associated with age: while young children often have asymptomatic infection, older children and adults commonly experience symptomatic disease [120,121]. The severity of disease and fatal outcomes are higher in older age groups. Infected children under 6 years are more likely to not experience noticeable symptoms, and only 10% develop jaundice. Among older children and

adults, infection usually causes more severe symptoms, with jaundice occurring in more than 70% of cases. Due to the often asymptomatic or subclinical course of hepatitis A infection, estimated incidence rates are most probably an underestimation.

Ultimately, hepatitis A resolves completely in >99% of the cases, although a relapse of symptoms has been reported in 3%–20% of clinical cases [122]. The person who just recovered falls sick again with another acute episode which appears several weeks after the first bout and associated with re-elevation of ALT and viremia manifested by re-detection of HAV-RNA in blood and shedding of HAV in stools . Recovery is usually complete within 24 weeks[122]. Unlike hepatitis B and C, HAV infection does not cause chronic liver disease, but it can cause debilitating symptoms and fulminant hepatitis (acute liver failure) with a reported incidence of 0.015-0.5%. Finally, co- or super-infection of HAV with a number of viruses including HBV, HCV, HEV HIV and dengue virus, as well as HAV infection in patients with chronic non-viral liver disease, may affect the natural history of the basic disease and lead to liver failure.[123,124]

Overall, mortality due to fulminant hepatitis is rare. Following the massive outbreak of hepatitis A in Shanghai, there were 47 deaths among >300,000 infected patients [125]. However, most of those infected in this outbreak were relatively young and otherwise healthy adults. The estimated case-fatality ratio of hepatitis A varies with age and ranges from 0.1% among children <15 years of age to 0.3% among persons 15–39 years of age. In age groups > 50 years and older, case-fatality may rise to 1.8-5.4% [89,116,117,126-129]. Fulminant hepatitis is rare, but associated with high mortality especially when liver transplantation is out of reach. Reports from South America and the Republic of Korea have raised concern that the incidence of fulminant hepatitis A may be rising, in particular in children. Hepatitis A has been reported to be the main cause of fulminant hepatic failure in 58-61% of cases in children in Argentina. In Argentina in children aged 1-18 years, 0.4% of pediatric cases developed fulminant hepatitis, of which 60% were fatal [130-134]. A retrospective analysis in Brazil reported that 39% of fulminant hepatic failure cases in children were associated with hepatitis A infection. In a multicenter, prospective study on fulminant hepatic failure cases in Latin America 43% of the cases were associated with hepatitis A [135,136].

As a consequence, the incidence of fulminant hepatic failure caused by hepatitis A in children was used as one of the indicators to measure the impact of universal hepatitis A immunization in Latin American countries. In Argentina, since introduction of the universal hepatitis A immunization program in 2005, the percentage of fulminant hepatic failure caused by HAV decreased from 54.6% (1993-2005) to 27.7% (2005-2008) in the post-immunization period: the number of fulminant hepatic failure associated with hepatitis A decreased from 2005, with no cases reported from November 2006-December 2008 [137].

Hepatitis A mortality as a consequence of acute liver failure may reach 90% in adults and 74% in children who do not undergo liver transplantation although spontaneous recovery even at an advanced stage of liver failure is possible. With liver transplant, however, the survival rate in children with acute liver failure may reach 80% [138,139].

Immunosuppressed patients (HIV, chronic diseases) and patients with chronic liver disease are at increased risk of developing severe or fulminant hepatitis [140-142].

6. Current Strategies for Control and Prevention of Hepatitis A

Hepatitis A is a vaccine-preventable disease. Protection against HAV infection is afforded by: 1) Adequate sanitation and housing facilities as well as personal hygiene; 2) pre- and post- exposure passive prophylaxis with immune globulin (IG), or 3) pre- or post- exposure active immunization with an HAV vaccine.

*Adequate sanitation and personal hygiene***.** HAV is resistant to freezing, moderate heating, drying, and low pH, and persists in feces, sewage and soil for prolonged periods[27,112,143,144],.Suitable sanitation to prevent oral transmission through fecal-oral contact with food and water or by person to person contact are important means to control transmission of hepatitis A.

*Pre- and post-exposure prophylaxis with immune globulin***.** For more than five decades, human immune globulin (IG), manufactured by ethanol fractionation of pooled human plasma samples, has been used as an efficient means for short-term pre- and post-exposure prophylaxis against HAV infection[75,145,146]. Pre-exposure prophylaxis via intra-muscular (i.m.) injection of IG is effective within hours from injection for a period of 12-20 weeks at a dose of 0.02- 0.06ml/kg weight, respectively. Post-exposure prophylaxis (PEP) is also achieved within hours of

injection and is 80-90% effective when administered no later than 14 days post exposure[145]. Importantly, while IG may protect against symptoms of hepatitis A, it may not prevent infection, particularly when given following exposure. The mechanism(s) involved in IG protection of HAV-induced liver injury are not understood, but may involve post-endocytic neutralization of virus within hepatocytes, as alluded to above. In recent years, declining concentrations of anti-HAV (IgG) antibodies in plasma pools have raised concern regarding the efficacy of IG in PEP[147]. The safety profile of IG administration is excellent except for recipients with IgA deficiency. Co-administration of IG with vaccines such as measles, mumps rubella, varicella and active HAV vaccines may partially blunt or neutralize the immune response of the particular vaccine. Yet, simultaneous administration of IG with formalin inactivated HAV vaccines does not affect the short and medium-term protection against HAV[148]. The effective PEP of HAV infection with inactivated HAV vaccines (provided it is administered within two weeks from exposure), as well as the high cost of IG and the short-term protection afforded through passive immunization, has led to declining use of IG altogether.

Active immunization with hepatitis A vaccines. All hepatitis A vaccines are manufactured using HAV strains that have been adapted to growth in mammalian cell cultures. Cell culture adaptation generally leads to attenuation of the pathogenicity of the virus, and is associated with a small number of mutations in the nonstructural proteins of the virus[149], particularly in the 2B and 2C proteins, as shown for the formaldehyde, inactivated and the live attenuated vaccines developed in the Western Hemisphere and in China respectively[150,151]. Hepatitis A vaccine induced protection against HAV challenge is defined by convention at an anti-HAV (IgG) antibody level ranging between 10-33 IU/ml, depending on immunoassay used.[152] However, protection may still be present at lower anti-HAV (IgG) levels as observed in IG recipients with undetectable antibody levels by a conventional immuno-assay[75]. Non- or low response to inactivated HAV vaccines, previously linked to lack of a putative HAV receptor on T cells, is very rare [153] (See reviews on the humoral and cellular immune responses to HAV vaccines[27,73,75,112,143-148,152,153]). Vaccine potency assays using a WHO international reference serum, and/or in-house assays, are used to compare vaccine biologic activity between manufacturers and results expressed in units or in microgram weight. Two types of hepatitis A vaccines are currently used worldwide:

a) Formalin-inactivated ("killed") hepatitis A virus vaccines. Monovalent inactivated vaccines are used in most countries i.m. (Table 1)[73,75]. All inactivated HAV vaccines contain aluminum hydroxide as an adjuvant except for one where virosomes are used as an immune stimulator. Inactivated HAV vaccines, stored at 2-8 ℃, have a shelf-life of 18-36 months, depending on manufacturer and are inter-changeable. Vaccines are licensed for use at the age of ≥12 months and are injected into the deltoid or thigh muscles in two doses at a 6 months interval (which can be extended to 12-36 months). HAV vaccines are freezing- sensitive as reported for two formaldehyde inactivated HAV vaccines containing aluminum hydroxide with a heat sensitivity ranging between 28 and 37°C[154].

Inactivated HAV vaccine can be administered with other vaccines[73]. In Argentina and some other countries, inactivated HAV vaccines are used in children as a single dose immunization strategy[155] . A similar one dose strategy is now being evaluated in China using a single attenuated, live HAV vaccine[156].

Inactivated combined vaccines against hepatitis A and B or hepatitis A and typhoid are available, mainly for travelers[73,75,157].

Cumulative experience based on use of hundreds of millions of doses of inactivated hepatitis A vaccines indicate an excellent safety record with acceptable reactogenicity and very rare vaccine related serious adverse events, irrespective of adjuvant, manufacturer or schedule [73,75].

Table 1: Monovalent-formalin inactivated HAV vaccines *,#

Attenuated HAV strain	Trade Name	Adjuvant	HAV Antigen Dose/injection		Manufacturers
			Pediatric	Adult	
HM-175	HAVRIX®	Alum hydroxide	720 EU	1440 EU	GSK
CR-326	VAQTA®	Alum hydroxide	25 U	50 U	MSD
GBM	AVAXIM®	Alum hydroxide	80 U	160 U	Aventis Pasteur
TZ84	HEALIVE®	Alum hydroxide	250 U	500U	Sinovac Biotech Co LTd
Lv-8	Weisairuian®	Alum Hydroxide	320 EU	640 EU	Inst Med Biol
YN5	Veraxim®	Alum hydroxide	800 EU	1600 EU	Shanghai Wison Bioengineering Inc
RG-SB	EPAXAL®	Virosomes	24 U	24 U	Crucell/Berna Biotech

*Modified and updated from references [75,158]. # Data on a Russian inactivated HAV vaccine Hep-A-in-Vav are unavailable in English[159]

b) Live attenuated hepatitis A vaccines. Live, attenuated HAV vaccines have been developed in China for subcutaneous (s.c.) injection in a freeze-dried form, and are also licensed in India, the Philippines, Thailand and Guatemala (Table 2)[73,75,158,160].These vaccines are usually employed in a single dose strategy and have a shelf life of 18 months[158]. In 2007, China introduced universal mass vaccination (UMV) targeting 18 months old toddlers. Half of the children received one s.c. dose of the live attenuated vaccine and half received two i.m. doses of a formalin-inactivated HAV vaccine, at a 6 months interval [158]. Seroconversion rates for the live vaccines were between 83-91%. Efficacy rates reached 90-95% for live and 95-100% for inactivated HAV vaccines. Adverse events (AE) such as fever, rash and elevated transaminases were rare and recorded at 34 AEs /million vaccinees but with "anaphylaxis" in 9% and no fatal outcome[158]. Attenuated HAV was isolated from stool of 34 vaccinees and 53/75 contacts had evidence for mild HAV infection with vaccine virus shedding in stools, suggesting person-to-person transmission from vaccinees. No reversion of the attenuated virus to virulence has so far been reported. Comparative immunogenicity studies between live attenuated and inactivated vaccines suggest that although seroconversion rates seem to be slower in the former, both vaccines induce similar protection rates for at least 8 years[73].

Table 2: Live attenuated hepatitis A vaccines**

Attenuated HAV strain	Name	Adjuvant	HAV Antigen Dose/injection		Manufactures
			Pediatric	Adult	
H2	Freeze-dried live HAV vaccine	None	0.5 ml (6.5log $CCID_{50}$)	1.0 ml (6.5log $CCID_{50}$)	Zhejiang Pukang Biotech company
LA-1	HAVAC Freeze-dried live HAV vaccine	None		1.0 ml (6.5log $CCID_{50}$)	Changchun Inst of Biologic Products

**Modified from reference[158]

Strategies for pre-exposure prophylaxis of hepatitis A. Several strategies exist for protection of individuals prior to exposure to the virus.

a) Targeted vaccination of populations at risk. Immunization strategies for prevention of hepatitis A depend on the epidemiology of HAV infection in particular geographic regions worldwide. Following the licensure of the first HAV vaccines, immunization was initially recommended for individuals at high risk for HAV infection (Table 3). Such a strategy for protection of susceptible persons is still valid in regions with low or very high HAV endemicity but not in areas of intermediate endemicity and in those areas in transition. This approach has little if any impact on herd immunity.

Table 3: Example of risk groups for HAV vaccination

• Travelers from non-endemic to HAV endemic countries
• Family members and close contacts of an individual with acute hepatitis A
• Men who have sex with men (MSM)
• Patients with chronic liver disease
• Day care center staff
• Laboratory and sewage workers with potential risk
• Immune suppressed patients living in areas of intermediate HAV endemicity
• Users of illicit intra-venous drugs
• Food handlers
• Recipients of frequent blood products
• Military personnel from non-endemic countries deployed overseas
• Care takers of non-human primates

b) Regional mass vaccination of pediatric populations at risk. The impact of mass vaccination against hepatitis A of susceptible children in communities at risk, was verified in a number of demonstration projects in Alaska, in American Indians, in Puglia (Italy), Catalonia (Spain), in Belarus and in Australia, leading to an up to 97% decline in the reported HAV incidence in the particular region[75]. The cumulative experience on immunogenicity and safety of inactivated HAV vaccines as well as reported cost-effectiveness of the intervention [75,161,162], led to introduction of universal mass vaccination (UMV) in babies, 12 months and older in several countries.

c) Universal mass vaccination with a two-dose inactivated vaccine regimen. Based on epidemiologic evidence that transmission of HAV mainly occurs through virus shedding by infected toddlers, Israel was the first country to introduce UMV in 18 month-old babies in 1999, using a two i.m. injection schedule, given at a 6 month interval[163]. At a vaccination coverage of 90 and 85% for the first and second dose, the overall reported incidence of HAV infection declined from 33-70 cases/100,000

between 1992-1998, to 2.5/100,000 cases in 2002. Long-term surveillance data confirm that this decline remained stable for 16 years post-introduction of UMV (Fig. 7)[163-165]. Thus, immunization of ~3% of the population annually, most likely was the reason for a marked decrease in attack rates of HAV infection within 3 years of initiation in all age groups and a shift from a state of HAV intermediate endemicity to very low endemicity.

In 1999, the US Advisory Committee on Immunization Practices (ACIP) recommended introduction of UMV in 11 states with a reported annual incidence of acute hepatitis A >20cases/100,000. This strategy led to a rapid decline of acute hepatitis A below 5/100,000 in all age groups despite a relative low coverage rate.

Following the success of the above programs, the WHO recommended in 2012 the integration of UMV against HAV infection in national immunization plans for children ≥1year in regions with declining incidence and epidemiologic shift from high to intermediate endemicity[73]. Consequently, 9 more countries have reached so far a decision to introduce UMV against hepatitis A in young children.

A recent survey has analyzed the impact of such UMV programs on the incidence of hepatitis A as reported in studies conducted in Argentina, China, Greece, Israel Panama, the US and Uruguay[166]. All except one study[167], reported a significant drop in the incidence of acute hepatitis A following introduction of UMV. Declines were documented irrespective of age at first vaccine dose (12-24 months), vaccine brand or vaccine coverage (range 25-96.8%). This decline in incidence was documented in vaccinated as well as non-vaccinated individuals in most age groups, confirming the original observation from Israel and elsewhere regarding the vaccine induced herd immunity in susceptible and non-vaccinated subjects[102,163]. Furthermore, vaccine induced long-term immune memory against hepatitis A was documented up to 20 years following a two dose vaccine schedule in 87-100% of vaccinees[103,105,168]. Grading of scientific evidence for long-term protection against hepatitis A induced by inactivated (IIIa) and live attenuated hepatitis A vaccines (IIIb); and evidence of a population impact of hepatitis A immunization programmes (inactivated or live vaccines) on hepatitis A morbidity (IIIc) and mortality (IIId) is available at http://www.who.int/entity/immunization/position_papers/hepatitisA_grad_long-term.pdf

Other indirect effects of UMV included a drop in hospitalization rates for hepatitis A in Greece; a decline in the reports on acute hepatitis A in day-care centers in Israel [169] as well as a reduction in HAV-associated fulminant hepatitis and age-adjusted mortality and liver transplantation in Argentina[137,166]. Finally, it seems that the cyclic epidemic peaks of acute hepatitis A are also dwindling. All in all, the overall decline in the incidence of HAV infection is most probably even higher as reported in the above studies, taking into account the low reporting rates of hepatitis A and the estimated large proportion of asymptomatic patients who got infected.

d) Single-dose UMV with inactivated or a live attenuated hepatitis A vaccines

High immunogenicity of inactivated hepatitis A vaccine has been shown following injection of the priming dose[170-172].

In 2005, the public health authorities in Argentina started a pioneering universal immunization program in 12 month old babies using a single inactivated vaccine dose protocol. The cost saving decision[173] was based on a prediction that immune memory would afford protection against HAV following administration of a single vaccine dose, and that this would be sufficient for protection in case of future exposure to wild-type HAV[99,100,174]. Following introduction of the program, the incidence of reported acute hepatitis A in Argentina, which fluctuated between 70.5-173.8 cases/100,000 between 1995-2004, dropped rapidly to ~10 cases/100,000 in all age groups, representing a >80% decrease in incidence[155,175].(Fig. 8). Moreover, the incidence of fulminant hepatitis A as well as referral for liver transplantation substantially declined subsequent to introduction of this single dose immunization schedule[137]. At a median post-vaccination interval of 7.7 years (up to 9.2 years), 97.4% of vaccinees maintained protective levels of anti-HAV (IgG) antibodies[155].

The short and medium-range effectiveness of a single dose immunization strategy has been confirmed and shown to be independent of the use of an inactivated or live attenuated vaccine, or adjuvant type, in subsequent studies conducted in Nicaragua[98],Korea[176], India[177], and China[156]. However, it remains to be seen whether a single dose immunization policy will indeed provide long lasting protection against hepatitis A and whether a booster dose will be required eventually. Importantly, the anti-HAV (IgG) antibody geometric mean titer concentration (GMC) at 5 years post vaccination with 2 vaccine doses was shown to be higher than in recipients of a single vaccine dose. However, a single dose of HAV vaccine was already sufficient to promote HAV-specific immune memory cell responses similar to

that induced by natural infection. HAV-specific T cell immunity induced by primary vaccination persisted independently of the protective plasma antibody levels. Moreover, a recent review of 15 data sources concluded that "boosterability" following a single dose injection of an inactivated HAV vaccine may last as long as 11 years and possibly longer[100]. Thus, in view of the high immunogenicity of HAV vaccines and given the rapid impact already observed in Argentina, the probability of acquiring symptomatic or even asymptomatic natural HAV infection in recipients of a single inactivated vaccine dose appears to be quite low.

Post-exposure prophylaxis against hepatitis A. Up until a decade ago, IG was the only recommended means for PEP against HAV. The declining anti-HAV (IgG) titers in donors of plasma pools used for preparation of IG, the short duration of protection, as well as increasing costs of IG led to the search for alternatives. Given the post-exposure incubation period of hepatitis A, which on average is about 28 days, but can be as low as 15 days, the time for pre-emptive intervention is limited to about two weeks. Data obtained from animal studies[178,179] and clinical trials[121,170,171,180] suggest that post-exposure immunization with an inactivated HAV vaccine is effective in prevention of infection, when given within 14 days of exposure. This was confirmed in a randomized controlled trial in Kazakhstan where 1090 HAV susceptible children and adults (age range 2-40 y, average 12 y) received either one dose of an inactivated HAV vaccine or IG, given mostly on the second week after exposure to confirmed cases of acute hepatitis A[68]. Symptomatic HAV infection occurred in only 3.3% and 4.4% of IG and inactivated vaccine recipients respectively (RR,1.35;95% confidence interval, 0.70 to 2.67), suggesting an estimated active vaccine efficacy in the range of 73-86%. These results, paved the road for the US CDC to recommend the introduction of PEP with vaccine instead of IG in 2-40 year-old exposed individuals[181]. Several reports confirmed the effectiveness of PEP using an inactivated HAV vaccine[171,182-185]. Although controlled clinical trials have not been published in subjects aged 41 years and older, clinical experience from previous trials suggest that such a policy will also be effective in individuals older than 40 years old[186]. PEP through active vaccination is restricted to formalin-inactivated vaccines and should not be used with live attenuated vaccines. Grading of scientific evidence for post-exposure efficacy of inactivated hepatitis A vaccines against hepatitis A as compared with no intervention is available at:

http://www.who.int/entity/immunization/position_papers/hepatitisA_grad_post_exposure.pdf[73].

7. Conclusion

HAV is an ancient virus that has long afflicted human populations. Recent virologic studies have shed new light on the structure, evolution, molecular virology, and pathobiology of this unusual picornavirus that exists in both quasi-enveloped and naked, nonenveloped infectious forms. Despite development of highly efficacious and safe hepatitis A vaccines which have been available for more than 20 years, hepatitis A remains a frequent and debilitating disease affecting millions of individuals annually. The continuous worldwide improvement in sanitary and socio-economic conditions and the introduction of universal mass vaccination in some countries is leading to shifts in the epidemiology of hepatitis A. This differs from many other viruses in a way that decreases in HAV endemicity, i.e. from high to low endemicity, results in a growing population of susceptible individuals at risk to HAV infection and to an overall lower population immunity. At the same time, the increasing globalization of the world's food supply poses novel challenges for prevention of food-borne HAV infections.

Hepatitis A is a vaccine-preventable disease and universal immunization of infants can be an efficient means for control of the infection in the entire population, young and adults alike. In order to introduce appropriate HAV vaccination programs, detailed and up-to date sero-epidemiologic data are crucial as well as novel tools to evaluate sources of HAV immunity.

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Legends to Figures

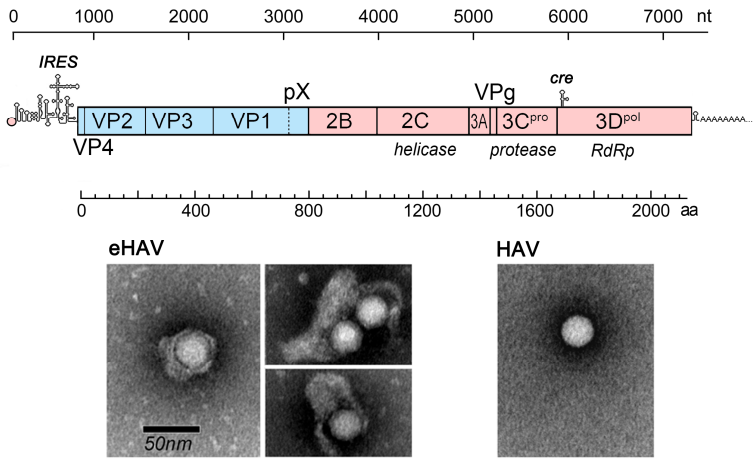

Figure 1. (top) Organization of the ~7.2 kb HAV genome. The open reading frame is shown as a box, flanked by the 5' and 3' UTRs. The 5'UTR is covalently linked to a small viral protein, VPg (or 3B), the protein primer for RNA synthesis at its 5' end, and contains an IRES the directs translation of the downstream ORF. The 3'UTR terminates in a 3' poly-A tail. Individual viral proteins are processed from the polyprotein as described in the text. With the exception of the VP0 (VP4-VP2) maturation cleavage and VP1-2A cleavage, all are mediated by $3C^{pro}$, a cysteine protease and the only protease expressed by the virus. (bottom) Transmission electron micrographs of eHAV and naked HAV particles found in the supernatant media of infected cell cultures (reproduced from Feng et al.[16])

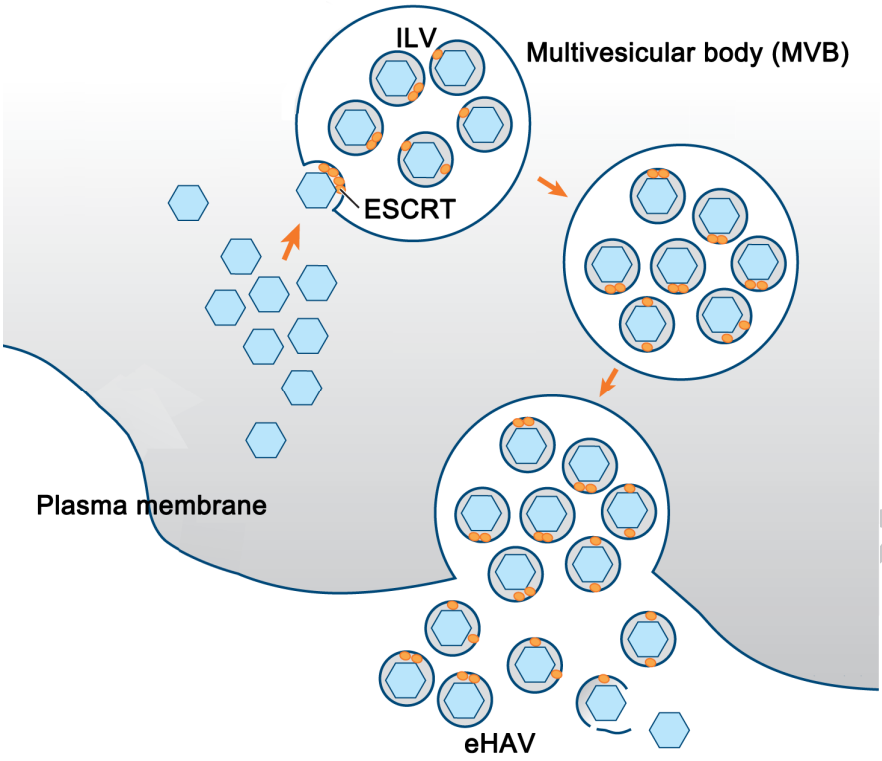

Figure 2. eHAV biogenesis. Assembled HAV capsids are recruited to ESCRT by interactions of the VP2 capsid protein with ALIX, and bud into late endosomes to form mutli-vesicular bodies (MVBs). Fusion of the limiting membrane of the MVB with the plasma membrane releases quasi-enveloped eHAV virions to the extracellular space. ILV = intralumenal vesicle.

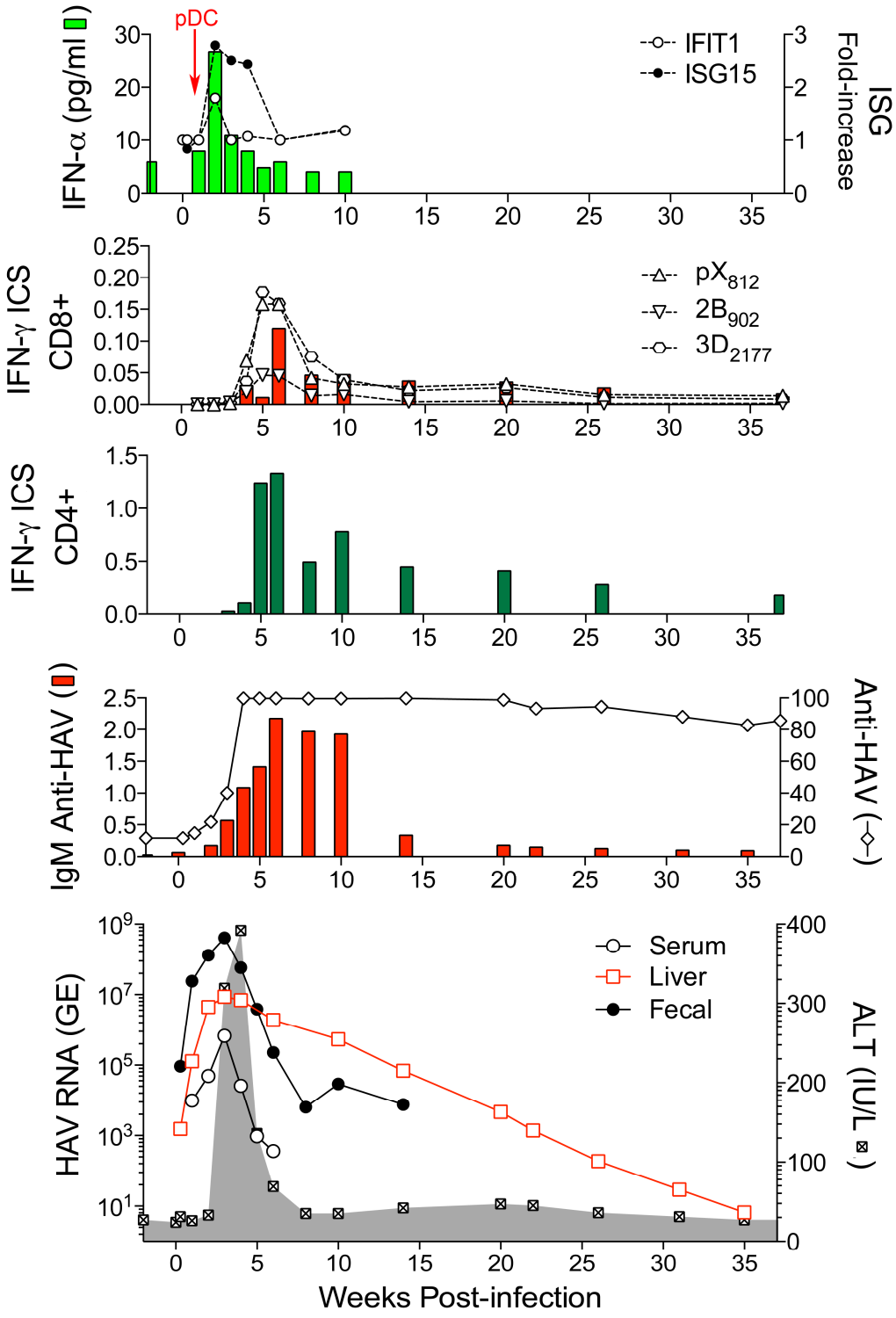

Figure 3. HAV pathogenesis in an experimentally infected chimpanzee, showing from the top: early but weak type 1 interferon and ISG responses associated with the transient appearance of pDCs in the liver; peripheral blood CD8+ and CD4+

T cell responses determined by intracellular cytokine staining when stimulated with HAV peptides, and CD8+ T cell frequencies determined by staining with three tetramers; IgM and IgG antibody responses determined by ELISA; and, viral RNA (GE=genome equivalents) detected in serum, feces, and liver. Reproduced from[70]

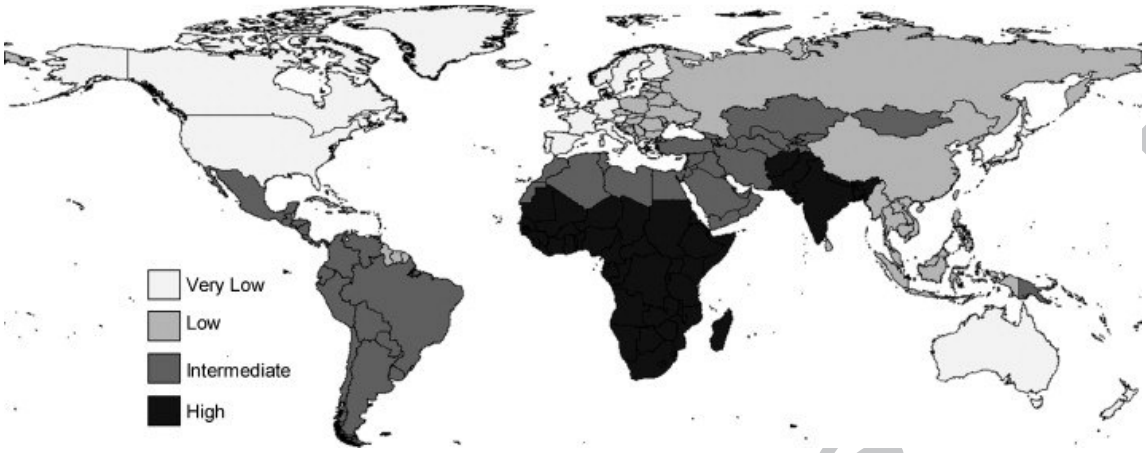

Figure 4 new. Global risk map of HAV immunity in 2005. Reproduced with permission from [77]

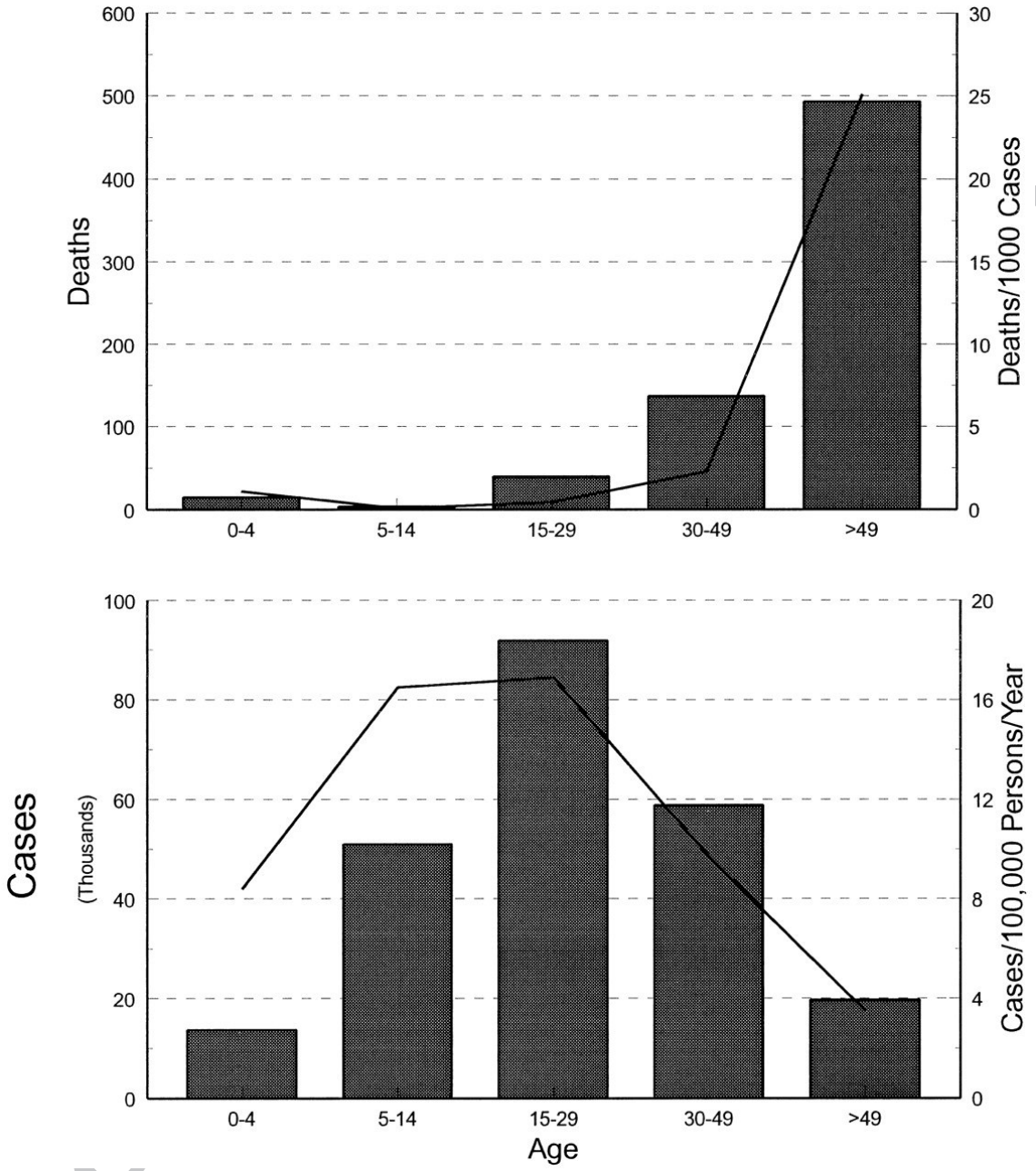

Figure 5. Age distribution of reported deaths from (n = 689, upper panel) and reported cases of (n = 235 153, lower panel) hepatitis A within the US, 1983–1991 (2). Bars represent reported deaths or cases (left ordinate), lines represent incidence rates (right ordinate). Reprinted with permission from [90]

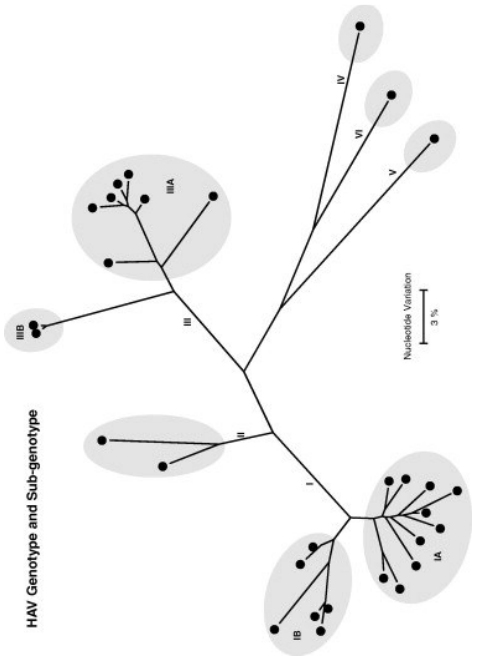

Figure 6- New: HAV genotype classification. Phylogenetic analysis of the six currently recognized human HAV genotypes Reproduced with permission from[71]

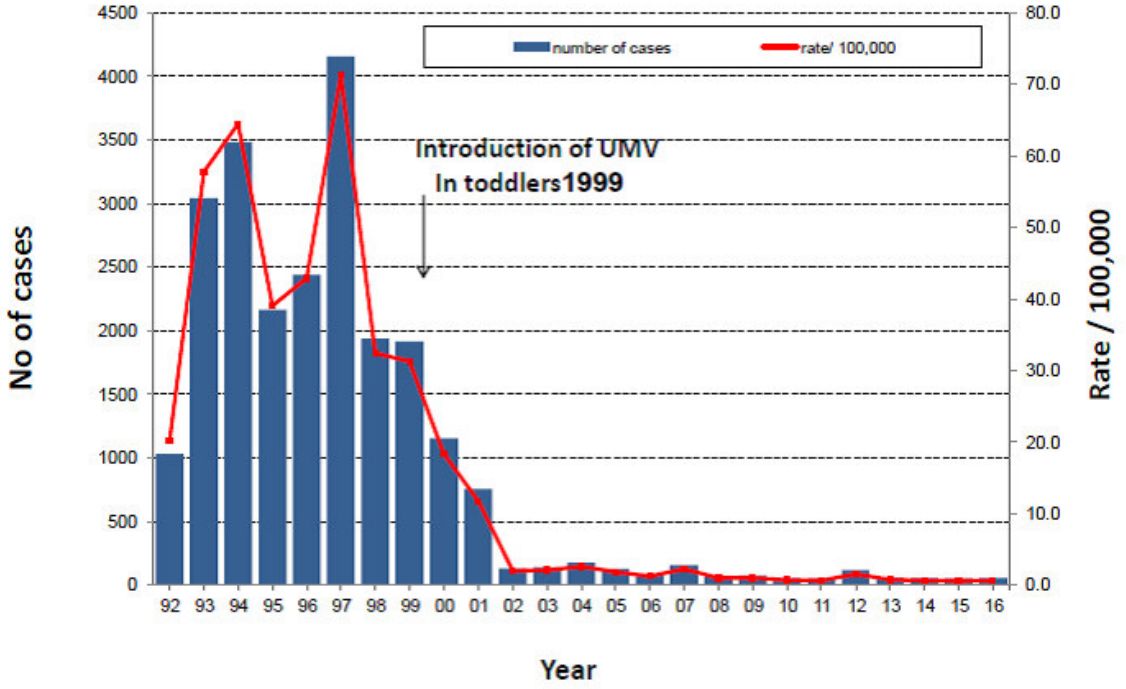

Figure 7 Incidence of acute hepatitis A in Israel between January 1992 and December 2016. UMV was started in 1999 with administration of an inactivated HAV vaccine at 18 and 24 months of age. Data collected through passive surveillance of the Israeli Ministry of Health (MOH). (Received by curtesy of Dr E. Anis, MOH, Israel).

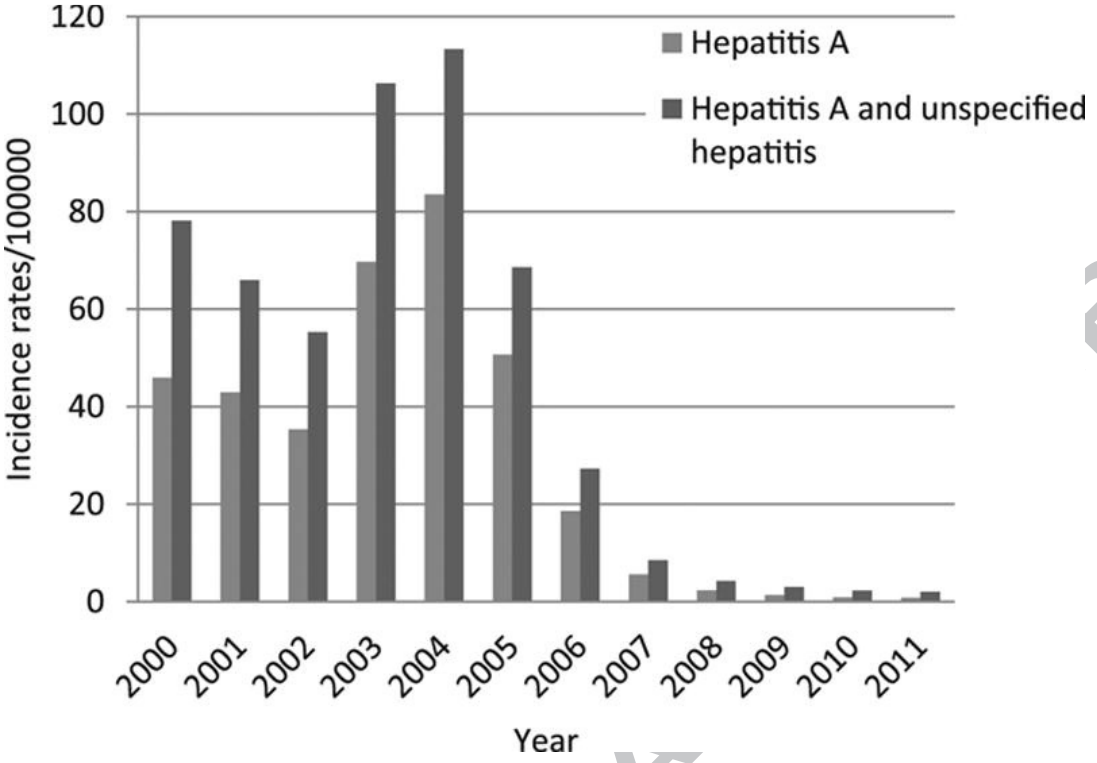

Figure 8: Impact of the single-dose immunization strategy against hepatitis A in Argentina. (Reproduced by permission from [175]).