# WHO Immunological Basis for Immunization Series

## Module 18: Hepatitis A
## Update 2019

Immunization, Vaccines and Biologicals

World Health Organization

# WHO Immunological Basis for Immunization Series

## Module 18: Hepatitis A
## Update 2019

**Immunization, Vaccines and Biologicals**

World Health Organization

**The immunological basis for immunization series: module 18: Hepatitis A (Immunological basis for immunization series ; module 18)**

**ISBN 978-92-51632-7**



**Suggested citation.** The immunological basis for immunization series: module 18: Hepatitis A. Geneva: World Health Organization; 2019 (Immunological basis for immunization series; module 18). Licence: *CC BY-NC-SA 3.0 IGO*.

**Cataloguing-in-Publication (CIP) data**. CIP data are available at *http://apps.who.int/iris*.

**Sales, rights and licensing.** To purchase WHO publications, see *http://apps.who.int/bookorders*. To submit requests for commercial use and queries on rights and licensing, see *http://www.who.int/about/licensing*.

# Contents

*Abbreviations and acronyms* ..... *v*

*Preface* ..... *vi*

*Acknowledgements* ..... *vii*

*Conflict of interest* ..... *viii*

**1. The hepatitis A virus, the infection, the disease and the vaccine** ..... **1**

1.1 Hepatitis A virus ..... 1
1.2 Epidemiology of hepatitis A virus infection ..... 3
1.3 Acute viral hepatitis A — clinical manifestations ..... 6
1.4 Hepatitis A vaccines ..... 7
1.5 Innate immune response in acute hepatitis A ..... 12
1.6 Humoral immune response in acute hepatitis A ..... 13
1.7 Cellular immune response in acute hepatitis A ..... 15

**2. Immune response to vaccination** ..... **16**

2.1 The humoral and cellular immune response to vaccination ..... 16
*2.2 Immunogenicity of hepatitis A vaccines and immune memory* ..... *19*

**3. Protection against hepatitis A** ..... **21**

3.1 Measurement of protection ..... 21
3.2 Passive prophylaxis with immune globulin ..... 22
3.3 Active immunization ..... 23

**4. Determinants of the immune response to immunization** ..... **25**

4.1 Risk groups and risk factors ..... 25
4.2 Interchangeability of HAV vaccines ..... 28
4.3 Immunization strategies for protection against hepatitis A ..... 28

**5. Safety of hepatitis A vaccines** ..... **36**

5.1 Monovalent hepatitis A vaccine ..... 38

**6. Conclusions** ..... **39**

**7. References** ..... **40**

# Abbreviations and acronyms

| | |
|---|---|
| ACIP | Advisory Committee on Immunization Practices (United States) |
| ALT | Alanine aminotransferase |
| ASP | Aspartate transaminase |
| BMI | Body mass index |
| CDC | Centers for Disease Control and Prevention (United States) |
| cDNA | Complementary deoxyribonucleic acid |
| DTaP | Diphtheria, tetanus, acellular pertussis vaccine |
| EIA | Enzyme-linked Immunoassay |
| ELISA | Enzyme-linked immunosorbent assay |
| EL.U | ELISA units |
| EPI | Expanded Programme on Immunization |
| GBD | Global burden of disease |
| GMC | Geometric mean concentration |
| HAART | Highly active antiretroviral therapy |
| HAV | Hepatitis A virus |
| eHAV | enveloped HAV |
| HAVCR1 | HAV with an attachment cellular receptor TIM 1 |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| Hib | Haemophilus influenzae type b |
| HIV | human immune deficiency virus |
| HLA | Histocompatibility leukocyte antigen |
| Ig | Immunoglobulin |
| MAVS | Mitochondrial antiviral signaling protein |
| MMR | Measles, mumps and rubella vaccine |
| MSM | Men who have sex with men |
| LAK | Lymphokine-activated killer cells |
| NK cells | Natural killer cells |
| PBMC | Peripheral blood mononuclear cells |
| RIFIT | Radioimmunofocus assay |
| RNA | Ribonucleic acid |
| TCID | Tissue culture-infective dose |
| UV | Universal vaccination |
| WHO | WHO World Health Organization |

# Preface

This module is part of the WHO series The immunological basis for immunization, which was initially developed in 1993 as a set of eight modules comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization (EPI) – i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance.

The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis for vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, published in the WHO vaccine position papers[1].

[1] See: *http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*, accessed 31 July 2018.

# Acknowledgements

The preparation of this publication was coordinated by the World Health Organization (WHO) Department of Immunization, Vaccines, and Biologicals. WHO thanks the donors whose unspecified financial support has made the production of this document possible.

This module was updated for WHO by **Daniel Shouval**[2], Professor of Medicine, Liver Unit, Hadassah-Hebrew University Medical Center, Jerusalem, Israel and **Pierre Van Damme**[3], Professor of Medicine, Faculty of Medicine & Health Science, Vaccine and Infectious Disease Institute, Centre for Evaluation of Vaccination, Universiteit Antwerpen, Belgium.

WHO also expresses its thanks to those who provided expert and technical reviews for the initial preparation of the module and the 2019 update: **Arthur L. Reingold** (Division of Epidemiology, University of California Berkeley), **Andreas Dotzauer** (Laboratory of Virus Research, University of Bremen), **Ettore Severi** (Epidemic Intelligence and Response, European Centre for Disease Prevention and Control (ECDC)) and **Yvan Hutin** (Global Hepatitis Programme, WHO).

[2] Liver Unit, Hadassah – Hebrew University Hospital.Ein-Kerem, P.O. Box 12000, Jerusalem 91120, Israel. *Shouval@hadassah.org.il*

[3] WHO Collaborating Centre for Prevention and Control of Infectious Diseases, Vaccine & Infectious Disease Institute, Centre for the Evaluation of Vaccination, University of Antwerp. Universiteitsplein 1- 2610, Wilrijk, Antwerpen, Belgium.
*pierre.vandamme@uantwerpen.be*

# Conflict of interest

**Daniel Shouval** co-authored a publication with employees of GlaxoSmithKline:

Stuurman AL, Marano C, Bunge EM, De Moerlooze L, Shouval D. Impact of universal mass vaccination with monovalent inactivated hepatitis A vaccines – a systematic review. Hum Vaccin Immunother. 2017;13(3):724–36.

This interest was assessed as personal, specific and financially insignificant.[4]

**Pierre Van Damme:** The University of Antwerp (Belgium) obtains research grants from vaccine manufacturers for the conduct of vaccine trials by the clinical trial team within the Centre for the Evaluation of Vaccination (led by Pierre Van Damme), and for the organization of the meetings and activities of the Viral Hepatitis Prevention Board. Some of the clinical trials reports were published in co-authorship with employees of the respective companies.

This interest was assessed as non-personal, non-specific and financially significant.

[4] According to WHO's Guidelines for Declaration of Interests (WHO expert), an interest is considered "personal" if it generates financial or nonfinancial gain to the expert, such as consulting income or a patent. "Specificity" indicates whether the declared interest is a subject matter of the meeting or work to be undertaken. An interest has "financial significance" if the honoraria, consultancy fees or other received funding, including those received by expert's organization, from any single vaccine manufacturer or other vaccine-related company exceeds US$ 5000 in a calendar year. Likewise, a shareholding in any one vaccine manufacturer or other vaccine-related company in excess of US$ 1000 would also constitute a "significant shareholding".

# 1. The hepatitis A virus, the infection, the disease and the vaccine

## 1.1 Hepatitis A virus

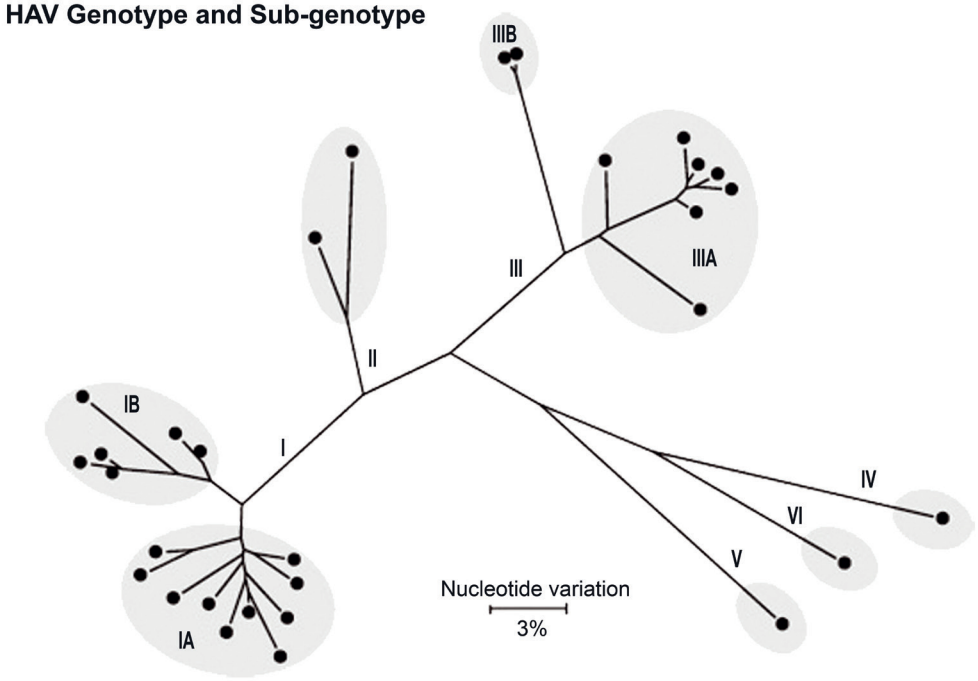
The hepatitis A virus (HAV) has been infecting humans for centuries [2,3]. The distinction between HAV and non-HAV viruses involved in clinical hepatitis was originally made in the 1940s [4]. HAV was first identified in 1973 through immune electron microscopy [5]. Today, it is classified as hepatovirus genus within the Picornaviridae family. HAV is a 27–32 nm, non-enveloped, icosahedral positive single-stranded linear ribonucleic acid (RNA) virus with a ~7.5 kb genome [6,7,8,9]. The genome contains three regions, namely: a 5' untranslated region with 734–742 nucleotides, a single open reading frame, and a 3' non-coding region of 40–80 nucleotides. See reviews in references [8,9]. There are two forms of infectious HAV – naked, non-enveloped virions containing the HAV RNA genome, shed in faeces and detectable by capsid antigen ELISA [10,11], and quasi-enveloped virions (eHAV) which are released non-lytically from infected cells as an enveloped form and are undetectable by the same ELISA [12]. eHAV is found in the blood of HAV-infected patients as well as in supernatants of HAV-infected cell lines. HAV is resistant to low pH and heat (60°C for 60 minutes) as well to freezing temperatures but becomes inactivated at 81°C for 10 minutes [13–15]. The virus may persist in faeces and soil for a prolonged period [13,15,16].

The exact pathway of eHAV through the gut wall is not fully known. Infection usually occurs through ingestion of HAV-contaminated food or fluid, with HAV passing through intestinal epithelial crypt cells, reaching the liver via the portal blood and being secreted into the bile. On the basis of the detection of HAV antigen in epithelial crypt cells in the gut in experimentally infected owl monkeys, HAV replication in the gut mucosa is presumed but not proven beyond doubt [17].

HAV has a special tropism for liver cells but is non-cytopathic; liver cell injury occurs through an immune-mediated mechanism. Several hypotheses address the mechanism(s) involved in cell entry of HAV through the hepatocyte membrane. These include the formation of virus-IgA complexes and uptake through the asialoglycoprotein receptor (such HAV-IgA complexes are able to pass into blood after oral uptake) [18]. Furthermore, interaction of HAV with an attachment cellular receptor TIM 1 (HAVCR1) expressed on the hepatocyte surface facilitates hepatocyte membrane penetration [10,19] . HAVCR1 may not be the only or essential entry factor and the virus may enter the liver cell through other or additional mechanisms [20]. Cell wall penetration seems to occur via endocytosis followed by removal of membranes around the capsid by bile acids which expose the virus to neutralizing antibodies when present. The virus replicates in the liver and is then shed into the bile and faeces and to a lesser degree into the bloodstream. Free or IgA-HAV immune complexes reach the small intestine.

Upon hepatocyte cell entry, host cell ribosomes bind to the viral uncoated RNA. HAV-RNA is then translated into a major protein of 2225 amino acids. The HAV replication cycle commences with translation of the polyprotein. The polyprotein is processed co-translationally into 10 mature viral proteins. These include, from the N-terminus, four structural proteins that form the capsid – VP4, VP2, VP3 and VP1pX (numbered according to molecular mass) – and six nonstructural proteins that are essential for amplification of the RNA genome [9]. HAV RNA can be detected in body fluids and faeces by using nucleic acid amplification and sequencing techniques. Such methods, mainly used by research laboratories, have been utilized for studies on the genetic organization of HAV infection as well as for investigating outbreaks through phylogenetic analysis [6,7,8,9]. Seven HAV genotypes I-VII were originally identified among a global collection of HAV samples using a 168 nucleotide fragment containing the VP1/2A junction. These were than reclassified into six genotypes based on sequences derived from the complete VP1 region. Genotypes are defined by a sequence variability of ~15% in these regions while subgenotypes differ by 7.0–7.5%. Three genotypes, namely I–III, were identified in infected humans, while genotypes IV–VI have been found in infected nonhuman primates (Figure 1). All HAV genotypes share a common serotype, irrespective of their origin and whether they derive from wild-type or attenuated strains. Consequently, all HAV vaccines – irrespective of attenuated strain used for manufacturing – provide protection against HAV infection. The identification of the various HAV genotypes and subgenotypes has significantly enhanced the ability to investigate the molecular epidemiology of hepatitis A outbreaks. Phylogenetic analysis is particularly helpful in investigations of sources and transmission routes as well as in discrimination between outbreak and non-outbreak cases [6,8].

**Figure 1: HAV genotypes classification.**
**Phylogenetic analysis of the six currently recognized HAV genotypes**




*Reproduced by permission of Vaughan et al.[5]*

## 1.2 Epidemiology of hepatitis A virus infection

The epidemiology of hepatitis A in various geographic regions is changing worldwide [21–25]. A rise in the world population and improving socioeconomic conditions, as well as immunization campaigns, are divergent factors with an impact on the incidence of HAV infections. It is estimated that more than 100 million hepatitis A virus infections occur annually worldwide, resulting in 15 000 to 30 000 deaths per year. Most of the HAV burden occurs in low- and middle-income countries. According to a WHO assessment, some 1.5 million HAV infections are reported annually [1]. Estimates of HAV infections are often based either on modelling or on age-adjusted prevalence of anti-HAV (IgG) serology. There is no accurate figure of the number of new HAV infections worldwide because of the large number of asymptomatic infections in the younger population and an assumed underreporting of 80% and more [1,22,23,25-33].

In 2010, the WHO reported the results of a reassessment of the global burden of disease (GBD) for hepatitis A. As a first step, WHO reviewed 637 eligible reports out of 2932 papers pooled into 21 GBD regions,. Results revealed a global increase from 117 million infections in 1990 to 121 million infections in 2005 [1,22,23]. According to this assessment, an increase in incidence was observed in the age groups 2–14 years and 30 years and older. Deaths increased from 30 283 in 1990 to 35 245 in 2005 [23]. Globalization – manifested by increased travel from non-endemic to endemic regions, the import of contaminated food products from HAV-endemic countries and the presence of men who have sex with men (MSM) – contribute to an increased risk for acquiring HAV, especially in countries of low or intermediate HAV endemicity. On the other hand, improving socioeconomic conditions, safer water and food supplies, and universal vaccination programmes (albeit available so far in only a relatively small number of countries) contribute to decreased exposure to HAV but also to increased susceptibility to infection, especially in young and even middle-aged adults [21,25]. These phenomena create a shift in HAV exposure and, consequently, in the epidemiology of hepatitis A. This paradox is manifested in a decrease in HAV endemicity – i.e. from a high to an intermediate endemic situation – resulting in a growing population of susceptible persons at risk of contracting HAV infection (including children, adolescents and adults) and to lower population immunity. At the same time, the increasing likelihood of exposure at adult age will make a somewhat asymptomatic disease more visible with more pronounced symptomatology when contracted later in life. This shift in susceptibility is reflected in the higher number of symptomatic infections in the recent years.

There are two main types of sources of information that can be used to estimate the burden of disease associated with HAV infection: 1) serological surveys estimating the prevalence of past infections, and 2) reporting systems measuring the incidence of morbidity or mortality from acute hepatitis A disease.

1. ***Measurement of prevalence:*** The global spread of HAV infection can been assessed by monitoring overall and age-specific prevalence, thus also enabling indirect measurement of incidence rates. Historically, several classifications have been employed by public health agencies to estimate HAV prevalence. Prevalence has been classified into high (> 50% of population), intermediate (15–50%) and low levels (< 15%) of endemicity, based on detection of anti-HAV immunoglobulin G (IgG) antibodies in selected populations [32]. Another categorization based on seroprevalence data suggests classifying endemicity as high (≥ 90% by age 10 years) intermediate (≥ 50% by age 15 years, with < 90% by age 10 years), low (≥ 50% by age 30 years, with < 50% by age 15 years) and very low (< 50% by age 30 years). Recently, the European Centre for Disease Prevention and Control (ECDC) has defined HAV prevalence as very low (< 2 cases per 10 000), low (2–19 cases per 100 000), intermediate (20–199 cases per 100 000) and high (≥ 200 cases per 100 000).[a] This ECDC classification has been used in the present review to assess the susceptibility to HAV infection in Europe (see below). Age-specific prevalence is considered to be a more accurate marker to classify endemicity [1]. High endemicity of HAV infection is found in countries with poor sanitary and socioeconomic conditions, where infection typically occurs before the age of 5 years. Intermediate endemicity of HAV is typically found in countries in transition from a low socioeconomic status to improved housing and hygienic conditions, mainly in specific segments of the population. In such countries, the paediatric population may escape HAV infection in early childhood. As a result, susceptible individuals are infected by HAV later in life during outbreaks or following person-to-person contact. HAV infection in these populations is associated with a higher proportion of more severe clinical manifestations as compared to the subclinical presentations in babies. In countries with low HAV endemicity, the risk of acquiring HAV infection is low or very low for the general population, although this may be substantially higher in individuals belonging to specific risk groups (Table 1).

**Table 1. Risk groups for contracting hepatitis A**

| |
|---|
| • Travellers 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 |
| • Garbage and sewage workers |
| • Laboratory workers handling infected specimens |
| • Immune-suppressed patients living in areas of intermediate HAV endemicity |
| • People who inject drugs |
| • Food-handlers |
| • Frequent recipients of blood products |
| • Military personnel from non-endemic countries deployed overseas |
| • Caretakers of nonhuman primates |
| • Children of migrants from HAV-endemic countries |
| • Family members of adoptees from HAV-endemic regions |
| • Homeless and street persons |
| • Prisoners |

[a] See: *https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/hepatitis-a-virus-EU-EEA-1975-2014.pdf*, accessed 15 April 2019.

2. ***Measurement of incidence:*** Direct assessment of incidence by anti-HAV(IgM) testing requires adequate surveillance with reliable reporting which is often missing. Detection of anti-HAV(IgM) antibodies is not necessarily discriminatory between acute HAV infection and recent vaccination, although anti-HAV(IgM) titers are much lower in vaccinees as compared to patients recovering from acute hepatitis A [34]. Furthermore, anti-HAV(IgM) testing is not always available in countries that are endemic for HAV. When such data are available (i.e. in countries with improving socioeconomic and sanitary conditions as well as adequate serological testing facilities), endemicity to HAV can also be classified as high (> 150 cases/$10^5$), intermediate (15–150 cases/$10^5$), low (5–15 cases/$10^5$), or very low with an estimated incidence of 5 cases/$10^5$.[b]

As already indicated, an epidemiological shift from high to intermediate endemicity of HAV has been observed in many countries, especially those with improving socioeconomic and sanitary conditions [22]. This transition in endemicity often results in a paradoxical individual susceptibility to infection leading to an increase in disease incidence later in life, with consequent increased disease severity despite the presence of improved socioeconomic and sanitary conditions for part of the population [1,25,33].[b]

An example for the potential consequences of such a shift in endemicity was clearly observed in the clinical manifestation of the HAV infections during the massive outbreak of hepatitis A in Shanghai in 1988 where over 300 000 persons contracted HAV infection within a short period [35,36].

Up-to-date data on the GBD, based on age-adjusted seroprevalence, are still incomplete. Yet the increased incidence of HAV infection in adults is expected to have an impact on the magnitude and severity of clinical disease, as already reported from the Republic of Korea [37]. Furthermore, in countries in transition, pockets of intermediate endemicity may exist within the same areas of high endemicity, facilitating HAV exposure in still susceptible age groups. Finally, despite the observed low attack rates of clinical hepatitis, especially in areas of high endemicity but also in countries with intermediate endemicity in transition, HAV infection has been identified as a leading cause of fulminant hepatic failure in a growing number of countries including in Argentina [40,41], Brazil [42] and the Republic of Korea [38,39].

HAV transmission occurs mainly through common source outbreaks (such as frozen food and waterborne outbreaks) as well as through person-to-person contact via a faecal-oral route. Shellfish are able to ingest and concentrate HAV and, as a result, become a reservoir for the spread of the virus. HAV is very rarely transmitted through blood products or iatrogenic medical procedures. Risk groups in low and very low endemic settings include: populations of low socioeconomic status living in crowded conditions; household contacts of infected individuals; children visiting day-care centres and kindergartens; institutionalized patients; prisoners and related caretakers; sewage and garbage collection workers; parents in low endemicity countries of children newly adopted from countries with high or intermediate endemicity[43]; MSM; homeless persons; people who inject drugs; patients with chronic liver disease; immune-suppressed patients such as those with HIV; food handlers; caretakers of nonhuman primates and patients with blood-clotting disorders (Table 1).

[b] See: *https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/hepatitis-a-virus-EU-EEA-1975-2014.pdf*, accessed 15 April 2019.

Although sequencing and phylogenetic analysis have improved significantly, the accuracy in identification of the source and routes of HAV transmission [43,44] the source remains unidentified in over 50% of cases [45]. The constant changes in the epidemiology of HAV worldwide – especially, though not exclusively, in resource-poor countries in transition – justify continuous surveillance which will be essential for public health decision-makers [33,43,44].

## 1.3 Acute viral hepatitis A — clinical manifestations

Acute hepatitis A virus infection causes an acute necro-inflammatory process in the liver which normally resolves spontaneously without chronic sequelae. The median incubation period of acute HAV is usually about 28 days, ranging from 14 to 50 days. Symptoms include malaise, fatigue, anorexia, vomiting, abdominal discomfort, diarrhoea and, less commonly, fever, headaches, arthralgia and myalgia. Five clinical/subclinical patterns are recognized: 1) asymptomatic HAV infection, often present in children under the age of 5 years; 2) symptomatic HAV infection associated with hepatocellular injury manifested with high alanine aminotransferase/aspartate transaminase (ALT/AST) elevation with the appearance of dark urine and sometimes clay-coloured stools, often accompanied or followed by jaundice; 3) cholestatic hepatitis characterized by pruritus, prolonged rise of alkaline phosphates, gamma glutamyl transpeptidase, bilirubinemia and weight loss; 4) relapsing hepatitis A infection manifested by reappearance of the clinical, biochemical and virological markers of acute hepatitis A after initial resolution; and 5) fulminant hepatitis, which frequently resolves spontaneously but which occasionally may require liver transplantation.

Extra-hepatic manifestations of acute hepatitis A may occur; they include skin involvement (rash, vasculitis, cryoglobulinemia), pancreatitis, mononeuritis, meningo-encephalitis, Guillain–Barré syndrome, carditis, glomerulonephritis, pneumonitis, haemolysis (especially in patients with glucose-6 phosphate dehydrogenase syndrome) and aplastic anaemia. Other manifestations include prolonged fatigue, right upper quadrant discomfort, fat intolerance and indigestion, weight loss, emotional instability and prolonged indirect bilirubinaemia. Acute HAV infection resolves spontaneously in over 99% of infected persons. Relapsing hepatitis A with subsequent complete resolution has been reported in 3–20% of patients with clinical hepatitis [46,47,48]. Fulminant hepatitis is relatively rare, with a wide range of estimates. Some reports on incidence of fulminant hepatitis, liver failure and death are based on data obtained 20–30 years ago or on information obtained in non-endemic countries. Immune-suppressed patients and patients with chronic liver disease (i.e. chronic HCV infection with HAV super-infection) are at an increased risk of developing severe or fulminant hepatitis [49,50]. Before widespread use of HAV vaccines, the reported fatality rate of HAV-associated acute liver failure ranged between 0.015 and 0.5% in children and adults over 40 years of age, respectively [51,52]. According to a recent GAVI estimate, in 2015 HAV caused approximately 11 000 deaths, reflecting an 0.8% mortality rate. Following the massive outbreak of hepatitis A in Shanghai in 1988, 47 deaths were reported among more than 300 000 infected patients (a case fatality of 0.015 rising to 0.05 in patients with underlying chronic liver diseases, i.e. with persistent HCV infection). In this massive outbreak, fatality in hepatitis B surface antigen (HBsAg+) carriers infected with HAV reached 0.05%, as compared to 0.015% in non-HBV carriers [35]. In Western countries, reports suggest a case fatality rate of ~0.3–0.6% in age groups over 40 years. In age groups of 50 years and older, case fatality may rise to 1.8–5.4% (these data reported from the United Kingdom and the USA were obtained prior to introduction of liver transplantation for HAV-induced liver failure) [53–59].

Two reports from the Republic of Korea and the USA, based on a relatively small sample size, revealed a spontaneous recovery from fulminant HAV infection in 55–57% of patients with acute liver failure, while 31–38% underwent liver transplantation and 6–14% of non-transplanted patients died [60,61]. Although not confirmed, increased susceptibility for development of severe or fulminant hepatitis A has been linked to a mutation of the HAVCR1 receptor [62]. Reports from South America and the Republic of Korea have raised concerns that the incidence of fulminant hepatitis A may be rising, particularly in children. Before the introduction of universal HAV vaccination in Argentina, hepatitis A was reported to be the main cause of fulminant hepatic failure (58–61% of cases in children diagnosed with liver failure). Overall in Argentina, 0.4% of paediatric cases with acute HAV infection developed fulminant hepatitis, of which 60% were fatal [63]. A retrospective analysis in Brazil reported that 39% of fulminant hepatic failure cases in children were associated with hepatitis A infection. In a multicentre, prospective study on fulminant hepatic failure cases in Latin America, 43% of the cases were associated with hepatitis A [64,65].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. Indeed, introduction of infant immunization against HAV is leading to a decline in incidence of fulminant hepatitis A, as reported from Argentina and the Republic of Korea [38,39,41,42,66].

## 1.4 Hepatitis A vaccines

Prevention of HAV infection requires a broad spectrum of general measures of hygiene which are closely linked to the level of socioeconomic standards. These measures include the maintenance of adequate sanitation, access to clean irrigation and drinking water, effective supervision of food handlers and food products, and proper surveillance of nursing staff caring for toddlers. Not less important, hepatitis A is a vaccine preventable disease and thus, immunization is the most effective mean for prevention of hepatitis A.

### *1.4.1 Monovalent and combined hepatitis A vaccines*

Attenuation of HAV through serial propagation in tissue cell culture in 1979 [67] led to the development of several HAV vaccines that were tested initially in nonhuman primates and then in clinical trials in humans. Two types of HAV vaccines are currently used worldwide: 1) formaldehyde-inactivated hepatitis A virus vaccines are used in most countries (Table 2a) [68-72] and 2) live attenuated vaccines manufactured and mainly used in China [73–77] and sporadically in India in the private sector (Table 2b) [78].

## Table 2a. Monovalent hepatitis A vaccines with attenuated, formaldehyde-inactivated hepatitis A virus, available worldwide [77,258]

| Trade name | Attenuated HAV strain | Adjuvant | HAV antigen dose/injection | | Manufacturers | Reference |
|---|---|---|---|---|---|---|
| | | | Paediatric | Adult | | |
| HAVRIX® | HM-175 | Aluminium hydroxide | 720 EU | 1440 EU | GSK | 68 |
| VAQTA® | CR-326 | Aluminium hydroxide | 25 U | 50 U | MSD | 69 |
| AVAXIM® | GBM | Aluminium hydroxide | 80 U | 160 U | Sanofi Pasteur | 70 |
| HEALIVE | TZ84 | Aluminium hydroxide | 250 U | 500U | Sinovac Biotech Co,Ltd | 77 |
| Weisairuian | Lv-8 | Aluminium hydroxide | 320 EU | 640 EU | Inst. Of Medical biology, Chinese Academy of Medical Sciences | 77 |
| Aimugen | KRM003 | Aluminium hydroxide | 0.5mcg | 0.5mcg | Chemotherapeutic Research Inst. Japan | 77 |
| VERAXIMR | YN5 | Aluminium hydroxide | 800 EU | 1600 EU | Shanghai Wilson Bioengineering Inc | 77 |
| EPAXAL®* | RG-SB | Virosome | 24U | 24 U | Crucell/ Berna Biotech/ Janssen-Cilag Ltd | *71* |

* Source: *https://www.medicines.org.uk/emc/product/4035/smpc*, accessed 16 April 2019.

## Table 2b. Monovalent hepatitis A vaccines manufactured in China [77] (reproduced by permission Wang et al. [61,77])

| Type | Trade name | Attenuated HAV strain | Year licensed | | Adjuvant | HAV antigen dose/injection | | Manufacturers |
|---|---|---|---|---|---|---|---|---|
| | | | Liquid* | Freeze-dried | | Pediatric (age, 1.5–15 years) | Adult (age, ≥16 years) | |
| Live attenuated vaccine initially in liquid form, now freeze-dried; (the liquid form was removed from the market) | Weisairuiji, Biovac-A, Mevac-A | H2 | 1992 | 2003 | None | 1.0 mL (6.50lg CCID50) | | Institute of Medical Biology of the Chinese Academy of Medical Sciences, Kunming |
| | | | | | | 0.5 | 1.0 | Zheijiang Pukang Biotechnology Company Limited, Zhejiang; Academy of Medical Sciences, Hangzhou |
| | HAVAC | LA-1 | 1997 | 2000 | None | 1.0 mL (6.50lg CCID50) | | Changchun Institute of Biological Products |

* Not available at present

### 1.4.1.1 Formaldehyde-inactivated vaccines

Formaldehyde-inactivated HAV vaccines include the monovalent HAVRIX® [68], VAQTA® [69], EPAXAL® [71], AVAXIM® [70] and four inactivated vaccines which are available in China (TZ 84 HEALIVE®, Lv-8 Weisairuian, Aimugen and Veraxim) (Table 2a) [72,79,77].[c]

Monovalent inactivated HAV vaccines manufactured in the western hemisphere are available in paediatric (≥ 1 year old) and adult doses and are licensed for two intramuscular injections given 6 months apart. The interval between the two doses is, however, flexible and under certain circumstances can be extended to 18–36 months (Table 2a).

In addition to monovalent HAV vaccines, formaldehyde-inactivated combination vaccines have been developed in Europe, including TWINRIX® and AMBIRIX® (hepatitis A and B) [80–85], VIATIM®/VIVAXIM® and HEPATYRIX® (hepatitis A and typhoid) [86].

### 1.4.1.2 Live attenuated hepatitis A vaccines

In China, two live attenuated hepatitis A vaccines are available and are distributed under various trade names and provided in a freeze-dried form. These live attenuated vaccines contain the H2 strain under the trade names of WEISAIRUIJI, BIOVAC A, MEVAC A and the LA-1 strain (HAVAC) [74,75–77]. These vaccines are administered using a subcutaneous single-dose immunization schedule [77,87]. The vaccines, which have a suggested shelf-life of 18 months, were tested in clinical trials in China (Table 2b) [74,76,77].[a] Live attenuated HAV vaccines manufactured in China are also available in the, Guatemala, India, Philippines and Thailand.

Both the live attenuated vaccines administered subcutaneously as a single dose and as three formaldehyde-inactivated vaccines administered in two i.m. doses were tested in clinical trials in China and integrated into the Chinese Expanded Programme on Immunization (EPI) in 2007 [77]. Immunization with a single dose of the live attenuated H2 strain led to a median seroconversion rate of 94% at 2 months following injection of 6.5 log 10 cell culture infectious units and remaining at 91% at month 6. Recent reviews summarize the immunogenicity of six controlled clinical trials with the attenuated HAV vaccine conducted in children in China, reporting 72–97.9% seroconversion rates and a good safety profile[77,87]. However, surveillance provided evidence for horizontal transmission of the faecally-shedded attenuated virus by vaccinees with, so far, no signs for reversion to virulence or clinical significance. Comparative immunogenicity studies between formaldehyde-inactivated and live attenuated HAV vaccines suggest a somewhat slower seroconversion rate in the latter with similar long-term seroprotection rates for at least 14–15 years [88].

[c] See: h*ttp://www.who.int/wer/2010/wer8530/en/index.html*, accessed 15 April 2019.

### *1.4.2 Attenuated hepatitis A virus strains*

All HAV vaccines contain HAV antigens derived from virus attenuated through serial propagation in cell cultures. (Tables 2a and 2b). HAV has been adapted to grow in human and nonhuman mammalian cells, including fibroblasts, African green monkey kidney cells, fetal rhesus monkey kidney cells, and human fetal lung diploid fibroblasts. Adaptation of HAV to propagation in tissue culture and attenuation is associated with a number of mutations, particularly in the 2B and 2C proteins, generated through serial passage of wild-type HAV [89,90]. Cultured cells persistently infected with HAV produce relatively low amounts of viral antigen [6,7]. Comparison of the nucleotide sequence of complementary DNA (cDNA) cloned from wild-type virus (propagated in vivo in liver of marmosets) with attenuated HM-175/7 and MK-5 HAV strain (propagated in tissue culture) revealed only a small number of nucleotide changes. These were distributed throughout the genome, some being apparently associated with growth adaptation in culture and attenuation [91]. Limited information is available on the genomic organization of the Chinese attenuated strains of HAV as compared to strains propagated in Europe or the USA [92,93].

Most attenuated virus strains used for vaccine production in the western hemisphere are grown in human diploid MRC-5 fibroblasts, and the nucleotide and amino acid sequences of the virus are about 95% identical in different strains. Cell culture-derived HAV antigen is purified in a number of steps, including ultrafiltration and gel chromatography, inactivated by formaldehyde, and adsorbed to aluminium hydroxide (HAVRIX®, VAQTA®, AVAXIM®) or formulated in influenza-reconstituted virosomes (EPAXAL®) which substitute alum hydroxide as an immune-stimulating adjuvant. VAQTA®, HAVRIX®, EPAXAL®, HEALIVE® and the Chinese Lv-8 vaccine are at present preservative-free and some vaccines contain additional excipients.

### *1.4.3 Vaccine potency*

The biological activity of cell culture-derived virus, or its inactivated viral antigens, is determined by different methods for the various hepatitis A vaccines. Methods used to quantitate infectivity of HAV harvested from cell culture before inactivation include radioimmunofocus assay, fluorescent focus assay, in situ radioimmunoassay and in situ hybridization [6,7,94,95]. The inactivated antigen immune reactivity and dose are measured for HAVRIX® through an enzyme-linked immunosorbent assay (ELISA) using a standard reference and expressed in ELISA units (EL.U). Paediatric and adult formulations contain 720 EL.U in 0.5 mL and 1440 EL.U in 1.0 mL respectively. Antigen potency for VAQTA® is measured through an enzyme-linked immunoassay using a standard reference reagent in which 1 VAQTA® unit corresponds approximately to 1 ng of viral protein antigen confirmed by amino-acid analysis. Paediatric/adolescent and adult formulations of VAQTA® contain approximately 25 and 50 antigen units in 0.5 and 1.0 mL respectively. AVAXIM® is available at 80 and 160 antigen units for paediatric and adult populations respectively. Potency is measured by an in-house assay. EPAXAL®, the aluminium-free HAV vaccine formulated in virosomes, contains a dose of 12 and 24 international units (IU) of HAV antigen in 0.25mL and 0.5mL for paediatric and adult use respectively.

IU are measured by an ELISA using an international reference serum of 100 IU (NIBSC code #95/500). Paediatric and adult doses for HEALIVE® contain 250 U/0.5 mL and 500 U/1.0 mL respectively. Weisairuian, another inactivated HAV vaccine manufactured in China, contains 320 and 640 EU for paediatric and adult formulations respectively. Veraxim® is the third inactivated HAV vaccine manufactured in China (Table 2a). Recently, the European Pharmacopoeia has updated its biological reference preparation for measurement of in vitro non-adsorbed, inactivated HAV vaccine potency [96].

Two of the live freeze-dried HAV vaccines manufactured in China contain a 6.5 tissue culture-infecting dose ($TCID_{50}$) derived from the $H_2$ and LA1 attenuated HAV strains (Table 2b).

Hepatitis A virus vaccines should be refrigerated at 2–8 °C. Freezing should be avoided as this is expected to affect immunogenicity. The shelf-life for formaldehyde-inactivated hepatitis A vaccines manufactured in the western hemisphere ranges from 24 to 36 months, depending on the manufacturer and the time of storage at the recommended temperature. Furthermore, reactogenicity and immunogenicity of HAVRIX® stored at 37 °C for up to one week, and VAQTA® stored at 37 °C for up to 12 months, do not differ from what was reported for these vaccines when stored at 2–8 °C [55,97].

### *1.4.4 Immune response to "natural" wild-type HAV infection*

The mechanism of hepatic cell injury induced by HAV is partially understood. The virus is not directly cytolytic. Available evidence suggests that immune-mediated liver cell injury involves innate, adaptive and cellular immune responses to HAV [9,98,99]. It is not known why hepatocyte injury, and as a result also clinical symptoms, are less pronounced in children as compared to adults.

## 1.5 Innate immune response in acute hepatitis A

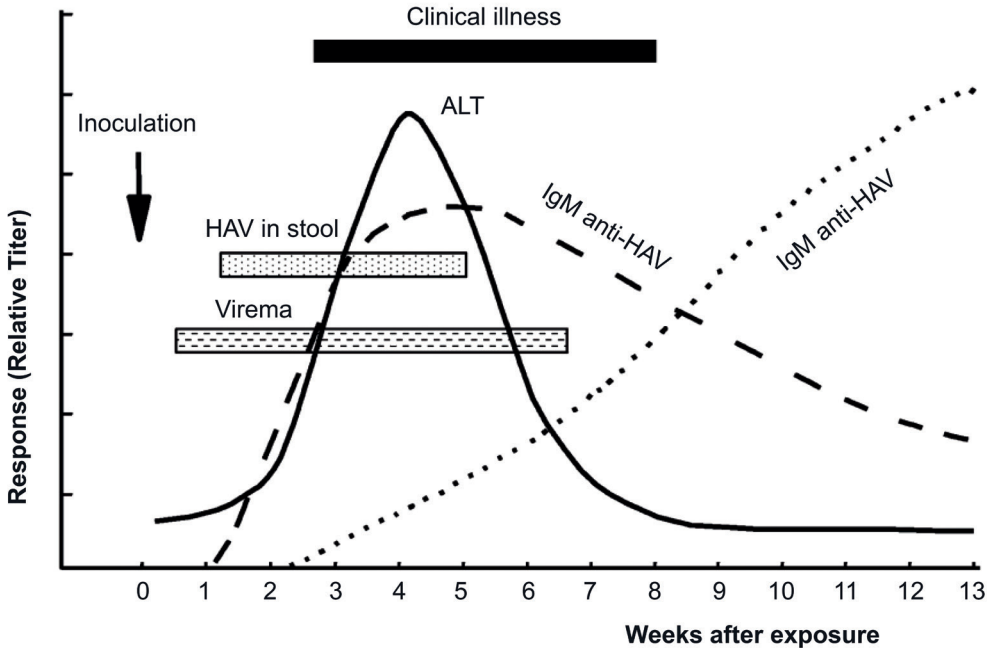
Limited information regarding innate immunity to HAV infection is derived from experimentally infected chimpanzees [100] and mice [9,101] (see review) [9]. HAV has been shown to actively suppress interferon responses through proteinases which proteolytically degraded mitochondrial antiviral signal protein (MAVS) and TRIF adaptor molecules involved in interferon induction. Thus, HAV has the capacity to evade MAVS mediated intra-hepatic interferon responses. A low interferon stimulating gene (ISG) response translates into resistance to develop an efficient interferon type I release despite high viraemia. Thus, blunted type I interferon production seems to influence the vigour and effectiveness of T-cell immunity against HAV and as a result contributes to the "stealth" property of the virus [100].

## 1.6 Humoral immune response in acute hepatitis A

The humoral immune response during acute hepatitis A includes a concerted generation of specific antibodies of the IGM, IgG and IgA isotypes directed against the HAV capsid. Diagnosis of acute hepatitis A is established through detection of IgM anti-HAV antibodies in patients with clinical/laboratory signs of hepatitis [1,102]. Post-infection and post-vaccination immunity is established through detection of total anti-HAV(IgG) antibodies [6,94,95]. Following acute HAV infection, cross-genotype neutralizing anti-HAV (IgG) antibodies react with an immune-dominant antigenic capsid epitope which is a conformational discontinuous complex structure formed from VP1, VP2 and VP3. Another putative conformational neutralizing epitope was recently located to amino acids on VP2 and VP3, providing lifelong protection against HAV re-infection. The mechanism involved in HAV neutralization is not completely understood. Most circulating HAV particles in blood are cloaked in host-cell membranes (eHAV-quasi-enveloped particles)[12]. These membranes are removed from the capsid and the intra-hepatic virus is then inactivated through post-endocytic neutralization by the specific antibodies [12]. Upregulation of specific immunoglobulin genes lead to expansion of multifunctional virus-neutralizing specific CD4+ T cells. Resolution of acute infection is also facilitated through release of antiviral cytokines which, together with the antibodies, lead to decline in intra-hepatic HAV-RNA [9]. The presence of total IgG anti-HAV antibodies in the absence of IgM anti-HAV antibodies signifies previous HAV infection with immunity against HAV (and exclusion of acute HAV infection). Commercially available enzyme-linked immunoassays are used for detection of IgM anti-HAV antibodies (directed against HAV capsid proteins) and for detection of total anti-HAV antibodies (IgG and IgM). IgM anti-HAV assays utilize the principle of direct binding of IgM anti-HAV in the test sample to anti-human IgM-coated matrix or particles. Competitive inhibition assays for measurement of total anti-HAV antibodies are available as qualitative or quantitative assays, the latter containing calibrators standardized against a WHO reference serum.

IgM, IgG and IgA anti-HAV antibodies appear shortly before, or during, the onset of symptoms [103]. Anti-HAV IgM antibodies are detectable in symptomatic and asymptomatic patients alike. In symptomatic patients, IgM anti-HAV antibodies appear within 5–10 days before symptoms, or at the early phase of alanine aminotransferase (ALT) elevation, and persist for about four months (range 30–420 days). In one study, HAV RNA was detected at an average of 17 days before the ALT peak, and viraemia persisted for an average of 79 days after the liver enzyme peak. The average duration of viraemia demonstrated in chimpanzees inoculated intravenously with HAV was 95 days (range 36–391 days) (Figure 2) [46,104,105]. In patients with relapsing hepatitis A (3–20% of patients), IgM anti-HAV, viraemia and shedding of HAV in the faeces may reappear intermittently for up to 6 months, and occasionally even longer [46,103,104,106]. False positive IgM anti-HAV may rarely be present in patients with hyperglobulinaemia [95,106]. In such cases, HAV RNA testing by PCR may facilitate the distinction between a positive and a false positive anti-HAV(IgM) result.

**Figure 2: Virological, immunological and biochemical events during the course of experimental hepatitis A virus infection in chimpanzees inoculated intravenously with human HAV, strain HLD2. ALT, alanine aminotransferase**




*Reproduced, by permission of the publisher, from Nainan et al.[6] and Margolis et al.[103]*

Total anti-HAV antibodies, often referred to as IgG anti-HAV, are detectable at the onset of symptoms. Their titre rises slowly parallel to the decrease in titre of anti-HAV IgM antibodies (Figure 2). IgG anti-HAV antibodies established through natural infection provide protection against re-challenge with hepatitis A virus and signify immunity against hepatitis A for life, irrespective of whether the infection was symptomatic or subclinical [6,107]. Immunity to HAV is established by convention once IgG anti-HAV antibodies rise to a titre above 10–20 mIU/mL, depending on the immunoassay used for detection. However, the absolute lower limit of protective antibody level has not been determined [108].

Qualitative assays for total anti-HAV antibodies are used for prevalence studies and, indirectly, for assessment of incidence when adjusted for age of detection. Anti-HAV(IgG) testing may also be used for assessing immunity prior to a decision to vaccinate as well as for confirming protection against HAV post-infection or post-vaccination. However, detection of anti-HAV(IgG) does not enable a clear distinction to be made between immunity generated by "natural", wild-type HAV infection, and vaccine-induced immunity. Such a differentiation may be possible in part through quantitative IgM and total anti-HAV measurements using modified, more sensitive immunoassays, since the humoral response to immunization is generally weaker compared to the response to wild-type HAV infection [94,109]. The inability to distinguish clearly between these two situations led to an attempt to develop an antibody assay against non-structural proteins such as the $3C^{pro}$ proteinase for differentiating the humoral immune response against replicating virus from the response to a killed-inactivated HAV vaccine [110–112]. However, these tests remain research tools only.

There are other sensitive, but also more time-consuming, methods compared to the commercially available assays for identification of neutralizing antibodies against hepatitis A. These assays, which are mainly used as research tools, include the radioimmunofocus inhibition assay (RIFIT), HAVARNA and radioimmunoprecipitation assay [94].

The role of secretory immunity in hepatitis A remains unclear. Anti-HAV(IgA) antibodies have been detected in the saliva of experimentally infected animals and humans [110,111]. Anti-HAV(IgA) antibodies appear in serum closely to generation of anti-HAV(IgM) antibodies. Such antibodies can also be found in some patients as virus-antibody complexes in stools of infected persons [113–115].

## 1.7 Cellular immune response in acute hepatitis A

Hepatocellular injury induced by HAV infection, has been linked to a cellular immune response presumably involved in the immunopathogenesis of the infection [116-24]. Despite the proven tropism of HAV for liver cells, the virus is not cytolytic and liver cell injury occurs through activation of HAV multispecific cytotoxic T cells[123]. Inflammatory cell infiltrates isolated from liver biopsies of patients with hepatitis A contain CD8-positive T cells which can specifically lyse hepatitis A virus-infected target cells in an histocompatibility leukocyte antigen (HLA) class I restricted manner [116]. Although there is only limited information on involvement of the innate immune system in HAV infection in humans (see section 2.1), there is evidence that secretion of interferon gamma by activated T cells may facilitate the expression of HLA class 1 determinants on the surface of infected liver cells. On the other hand, HAV is able to induce cleavage of MAVS and TRIF which are signaling molecules for induction of interferon responses [125,126]. Consequently, HAV is able to block the induction of beta interferon through interference with IRF-3 phosphorylation. Cytolytic T-cell epitopes residing on the structural proteins of HAV (i.e. the dominant epitopes in 2B, 2C and 3D) may be involved in cytolysis of HAV-infected hepatocytes [120,121,123]. Little is known about the role of T-helper cells in mounting an immune response to HAV. One putative CD4 T-cell helper lymphocyte epitope was identified on the VP3 102–121 sequence [118]. There is also some evidence that nonspecific immune mechanisms, including natural killer cells (NK) and lymphokine-activated killer cells (LAK), are involved in the induction of hepatocellular injury even before the initiation of cytotoxic T lymphocyte injury [119]. Finally, impaired function of CTL-suppressing CD4+/CD25+ regulatory T cells has been linked to the frequent resolution of acute hepatitis A with spontaneous recovery [122]. Thus, it appears that multiple immune mechanisms contribute to HAV induced acute liver injury and recovery [9].

# 2. Immune response to vaccination

Formaldehyde-inactivated hepatitis A vaccines are highly immunogenic and safe, providing rapid, protective immunity to hepatitis A. The extraordinary immunogenicity of these vaccines enables a flexible interval between the first and second dose. Thus, a booster dose is usually administered within 6–12 months after the priming injection. However, the interval may be extended to 18–36 months, depending on the vaccine type. Based on clinical data and mathematical modelling, protection against HAV is estimated to last for decades, and possibly a lifetime [127–129].

## 2.1 The humoral and cellular immune response to vaccination

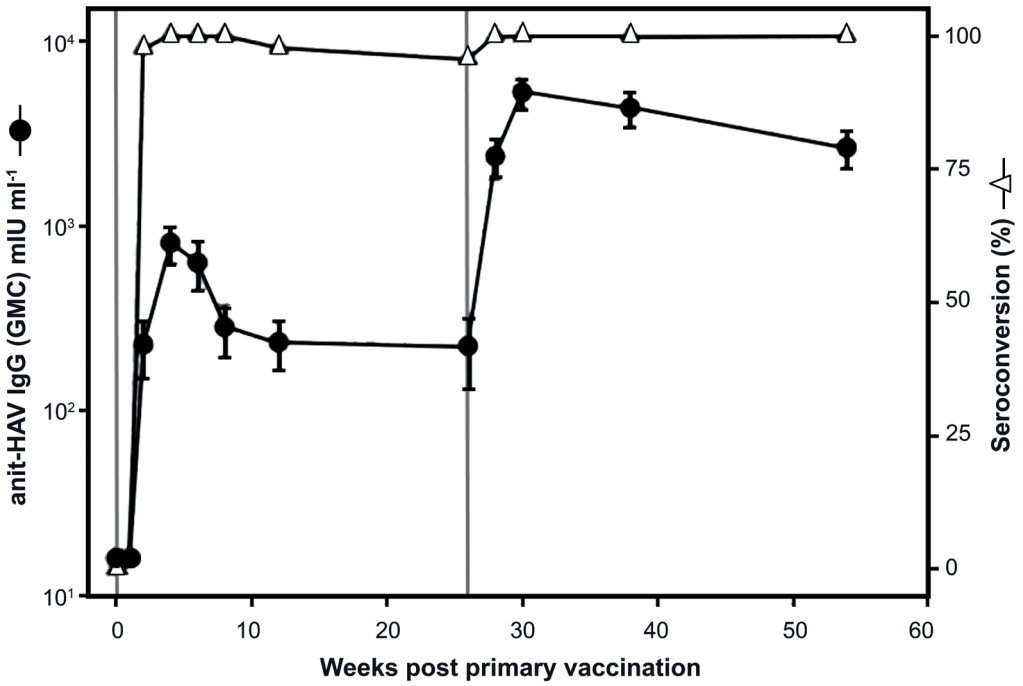
Infection with live, wild-type HAV involves virus replication in liver cells and is associated with an active cellular and humoral immune response against the virus which induces hepatocellular injury [94,116–122]. By contrast, viral replication does not occur after immunization with a killed HAV vaccine, and protection against HAV is primarily antibody-based [34,94,130]. Indeed, experience with immune globulin (Ig) and active immunization initially suggested that vaccine-induced protective immunity against hepatitis A is mainly humoral with little involvement of the cellular immune system [34,108,130]. Evidence has now been obtained to suggest that immunization against HAV with a killed vaccine also leads to a measurable cellular protective immune response, which is long-lasting for at least 6 years and may be boosted to revive the immune memory [131–135].

### *2.1.1 The humoral response*

Many studies have documented the rapid and effective humoral immune response to inactivated hepatitis A vaccines [34,70,94,127,130–139].

One example of careful prospective evaluation of the immune response to HAV vaccine was reported in 45 healthy vaccinees (mean age 28.4 years, 23 females) who received 1440 IU of HAVRIX® at 0 and 6 months [131] (Figure 3a). Seroprotection after the first dose was already documented in 97.7% of vaccinees by week 2, reaching 100% at week 4. Geometric mean concentration (GMC) of total anti-HAV antibodies (mainly IgG) at week 2 and 4 reached 211 and 812 mIU/mL respectively (range 117–5688 mIU/mL). At month 6, all vaccinees were still seropositive but antibody levels had dropped by 80%. However, a booster dose at month 6 led to a 24-fold rise in total anti-HAV levels, reaching a GMC of 5375 mIU/mL (range 745–34 245 mIU/mL). Similar results were obtained with other inactivated HAV vaccines, including VAQTA® [130], in a small-scale comparative randomized trial between HAVRIX® and VAQTA® [34], with AVAXIM® [70], EPAXAL® [71] and HEALIVE® [72].

**Figure 3a: Seroconversion rate (open triangles) and serum anti-HAV IgG responses expressed as geometric mean concentration (filled circles) in 45 adults receiving 1440 EU HAVRIX® at 0 and 26 weeks (vertical reference lines). Error bars indicate 95% confidence interval**



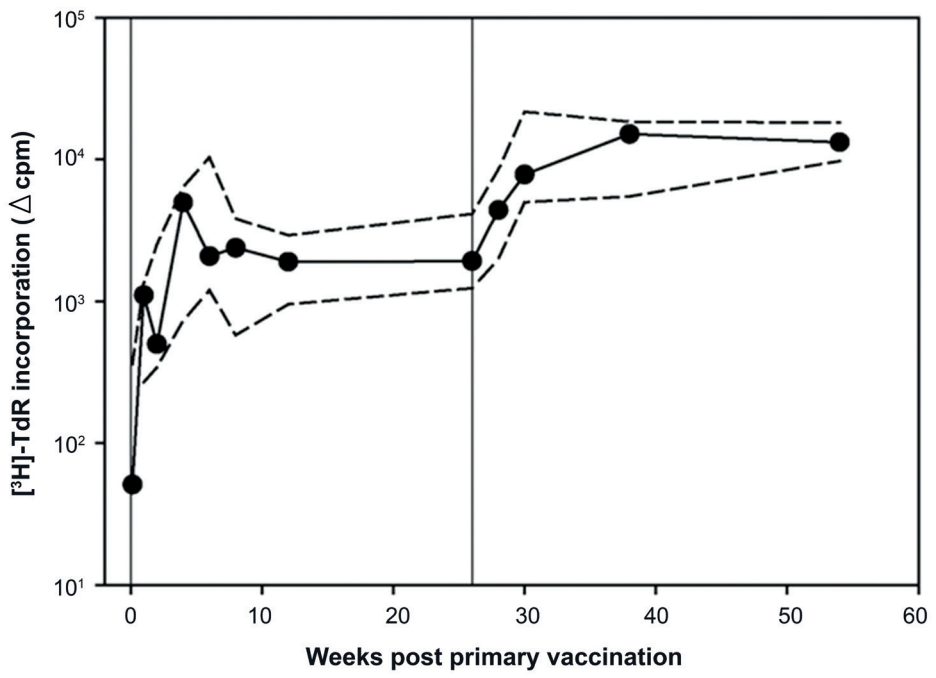

*Reproduced, by permission of Elsevier, from Schmidtke et al.*[131]

On the basis of this information, the humoral immune response to a single dose of an attenuated vaccine is slower compared to that of formaldehyde-inactivated vaccines. One month after a single-dose injection, 81% of 102 vaccinees had detectable anti-HAV (IgG) antibodies (GMC 56 mIU/mL) rising to a peak of 95% (GMC 131 mIU/mL) at two months. At 96 months, 72% of vaccinees were still anti-HAV (IgG) positive (GMC 89mIU/mL). A booster dose led to an anamnestic response in 100% of vaccinees (GMC 3133mIU/mL) [76]. Reports in the Chinese literature indicate seroconversion rates of 68–94% six weeks after single-dose immunization with the attenuated H2 strain and 57–100% at six months. Furthermore, anti-HAV (IgG) antibodies were still detectable in 72–88% of vaccinees at 15 years. It is, however, not known if these vaccinated individuals were naturally exposed to wild-type HAV during this period.

### *2.1.2 The cellular response*

Few studies have examined the cellular response to inactivated HAV vaccines [131–135]. Interferon gamma secretion as evidence for an HAV-specific proliferative T-cell response was documented in three studies [131–133]. In one study, a single 1440 EL.U dose of HAVRIX® induced a specific proliferative T-lymphocyte response in up to 100% of vaccinees, starting at day 7 post-injection [131] (Figure 3b). A 60% decline in cellular immune responses was observed by week 12 after primary immunization but rose over 7-fold after a booster dose at month 6. In another study of the innate immune system, production of interferon gamma and interleukin-10 by peripheral blood lymphocytes was documented by day 10 after immunization against HAV [135]. Garner-Spitzer and co-workers have documented a direct correlation between the concentration of cytokines IL-2, interferon gamma and IL-10 with anti-HAV antibody levels before and after booster vaccination [134]. These results provide evidence for the role of cytokines and T-helper cells in induction of protective immunity against hepatitis A through vaccination. More information on the cellular immune response in recipients of the live attenuated HAV vaccine is not yet available.

**Figure 3b: Median-specific proliferative response of peripheral blood mononuclear cells (PBMC) to inactivated hepatitis A virus in 15 adults receiving 1440 EU HAVRIX® at 0 and 26 weeks (vertical reference lines). Data are presented as Δcpm; upper and lower lines represent the upper and lower quartile ranges**




*Reproduced, by permission of Elsevier, from Schmidtke et al.[131]*

### *2.1.3 Summary*

In immune competent individuals, vaccination against hepatitis A using a two-dose schedule of formaldehyde-inactivated vaccines leads to a significant cellular and humoral immune response within two weeks of the primary injection, rising to 100% by week 4. A 60–80% decline in T-cell and B-cell responses within 24 weeks is reversed through a booster dose at week 24, leading to a significant rise in cellular and humoral immune response.

## *2.2 Immunogenicity of hepatitis A vaccines and immune memory*

The duration of protective immunity following wild-type HAV infection is thought to be lifelong [9,107,129]. Similarly, primary immunization with the first dose of inactivated hepatitis A vaccines provides the basis for long-term immunity – probably for decades [28,128,129,133], while non-response to immunization is extremely rare [134]. Inactivated hepatitis A vaccines are usually recommended using a 2-dose schedule, with a second dose given at 6–12 months after the priming dose for HAVRIX® and EPAXAL®, at 6–18 months for VAQTA® and AVAXIM® 80U, and at 6–36 months for AVAXIM® 160U. The interval between the priming vaccination and the second dose is flexible. This is particularly important for vaccinees who missed their second dose (e.g. international travellers). Furthermore, in children and young adults, up to 100% of vaccinees will seroconvert with anti-HAV IgG titres over 20 mIU/mL within 2–4 weeks of the first injection (Figure 3a).

In China, where live attenuated vaccines are used with a one-dose schedule, the persistence of anti-HAV(IgG) antibodies was documented after 15 years in 72–88% of subjects immunized with the H2 live attenuated HAV vaccine [74,77,141].

Early studies in chimpanzees immunized against hepatitis A with a formaldehyde-inactivated vaccine and challenged with wild-type HAV revealed that, even in the case of waning anti-HAV IgG antibodies at the time of HAV challenge, animals were protected against hepatitis A [136]. A specific gamma interferon-mediated T-cell immune response was documented in human vaccinees within 1.5–6 years of the completion of the original vaccine series [133]. In this study, a booster dose was given to 36 subjects 6 years after initially receiving two doses of HAVRIX®, 1440 EL.U at a 6–12-month interval. More than 50% of vaccinees had an HAV-specific proliferative T-cell response in vitro, providing evidence for prolonged immune memory induced through immunization. In another study, protective anti-HAV(IgG) were still detectable in 98% of 1016 travellers tested 10 years after immunization with 720 EL.U HAVRIX® [109]. Similar results of long-term persistence were recorded in 98% of Argentinean children 10 years after immunization with a two-dose vaccine schedule [142]. While cellular immune memory to HAV vaccines has so far been shown to persist for at least six years, available evidence already suggests that humoral immune memory will most probably persist for 2–3 decades or more, as demonstrated in follow-up studies and through mathematical modelling of antibody decay [28,137–139,143,144]. Challenge of vaccinees 12 years after completion of an HAV immunization series led to a robust anamnestic response, as manifested by a significant rise in anti-HAV antibody titre [143]. Furthermore, administration of a booster dose up to 6 years after one vaccine dose led to a 32–100-fold rise in anti-HAV IgG antibodies. A recent small-scale study compared HAV specific memory cellular responses between recipients of a single or two-dose inactivated HAV vaccine and patients following "natural" HAV infection [145].

Proliferation assays revealed that the first vaccine dose induces HAV-specific cellular responses similar to that induced by a second dose or by natural infection. Increase in the frequencies of classical memory B cells, TCD8 cells and central memory TCD4 and TCD8 cells were observed from the first to the second dose. This response was accompanied by increased IL-6, IL-10, TNF and interferon gamma cytokine levels after vaccination. The HAV-specific T-cell immunity induced by primary vaccination persisted independently of the protective plasma antibody level. These results suggest that a single dose of HAV vaccine promotes HAV-specific memory cell responses similar to that induced by a natural infection [145]. Thus, there is to date ample evidence to conclude that immune memory against hepatitis A, established through vaccination, will persist for decades in immune competent persons. The excellent record of immunogenicity of hepatitis A vaccines in children has led to the introduction of a single-dose immunization strategy in the routine childhood immunization programme in Argentina [146] and in other Latin American countries.

# 3. Protection against hepatitis A

Hepatitis A virus infection is a vaccine-preventable disease. General measures of protection include adequate personal hygiene, especially by food-handlers and by nursing staff at day-care centres, quality control and maintenance of a safe water and food supply, and proper sanitation. Specific measures include: 1) short-term pre- and post-exposure prophylaxis with immune serum globulin (Ig), if available; 2) long-term pre- and post-exposure active prophylaxis using formaldehyde-inactivated HAV vaccines; and 3) pre-exposure prevention using a live attenuated vaccine.

## 3.1 Measurement of protection

A positive (qualitative) test for total anti-HAV antibodies signifies immunity to hepatitis A. The lowest protective level against challenge with HAV is unknown. Clinical experience suggests that protection against hepatitis A following passive immunization with Ig or active vaccination may still be present even in the absence of detectable anti-HAV antibodies using standard immunoassays [55]. Different thresholds for protective total anti-HAV (mainly IgG) antibody levels have been suggested when studying individual HAV vaccines while using a WHO reference serum. The reported minimal serum levels of total anti-HAV antibodies required for protection against HAV in humans varied between 10–33mIU/mL, depending on the immunoassay used for detection and regardless of whether these antibodies emerged following "natural" wild-type HAV infection or appeared following vaccination [94,137]. Immunogenicity studies with VAQTA® have employed a modified radioimmunoassay with a minimal protective antibody level of ≥10 mIU/mL [130,138]. Early studies with HAVRIX® set the threshold of protection between 20 and 33 mIU/mL using an enzyme-linked immunoassay [139]. This threshold has been cut to 15 mIU/mL in recent years[147].

Low levels of anti-HAV IgM antibodies may be detectable by a conventional assay for a few weeks in ~20% of recipients of HAV vaccines [130]. Consequently, anti-HAV IgM antibody assays cannot be used to reliably distinguish between acute hepatitis A and anti-HAV response to vaccination.

## 3.2 Passive prophylaxis with immune globulin

Since the late 1940s administration of human immune serum globulin (Ig) has been considered as an efficient means of pre- and post-exposure prophylaxis against hepatitis A virus infection [108,148-150]. Immune globulin is prepared by ethanol fractionation from pooled human plasma [151] screened for hepatitis B and C, as well as for human immune deficiency virus (HIV). Historically, Ig contained very high concentrations of anti-HAV antibodies. However, in recent years, low titres of anti-HAV antibodies have been observed in plasma donors of Ig in the western hemisphere [152–154]. Immune globulin is administered through intramuscular injection. The protective efficacy of Ig against HAV infection is well documented [108,155,156]. The duration of protection is, however, limited to 12–20 weeks following administration of 0.02 and 0.06mL/kg weight respectively. Pre-exposure prophylaxis is achieved within hours of injection and is 80–90% effective when administered as close as possible to exposure and no later than 14 days [108].

Despite the proven efficacy of Ig, serum concentration of anti-HAV antibodies in Ig recipients for pre- or post-exposure prophylaxis against HAV infection is low [130] and is often below the detection limit of 15–20mIU/mL of commercially available assays [157]. The mechanism of both pre- and post-exposure protection against hepatitis A conferred by Ig is not fully established but it most probably involves neutralization of circulating virus and possibly prevention of uptake of virus through the gut mucosa and by hepatocytes. Evidence for the efficacy of Ig in pre- and post-exposure prevention of liver disease is well documented, provided that Ig is administered within two weeks of the latter. Yet, given the fact that circulating HAV is cloaked in membranes, it is not yet understood how anti-HAV(IgG) binds to HAV during the incubation and early period of infection. It is possible that contact between virus and antibody occurs following post-endocytic neutralization within endosomes or lysosomes after removal of the membrane from the viral capsid [9].

Administration of Ig is considered very safe but is contraindicated in patients with IgA deficiency who may develop an anaphylactic reaction. Interference with live attenuated vaccines such as those against measles, mumps, rubella (MMR) and varicella requires special caution. Co-administration of Ig with a hepatitis A vaccine may blunt the initial quantitative anti-HAV antibody response after the first vaccine dose [158,159]. However, this effect, which is similar to the effect of passively-transferred maternal anti-HAV antibodies, is of minor practical significance and apparently does not reduce the long-term immune memory. Furthermore, such vaccinees responded well to the booster dose given 6 months after the primary immunization. Additionally, although administration of Ig is highly efficacious for pre- and post-exposure short-term prophylaxis, the use of immune globulin worldwide is now declining for a number of reasons: 1) plasma pools used for manufacturing of Ig preparations increasingly fail to contain adequate amounts of anti-HAV (IgG) [152-154,160,161]; 2) the cost of specific HAV Ig preparations is high [160,162]; 3) there are limited stocks and therefore limited availability of Ig preparations (at short and even long notice); 4) Ig mediated protection against HAV infection lasts only several months as compared to hepatitis A vaccines [108]; and 5) inactivated hepatitis A vaccines have already been shown to induce very rapid protection against HAV following the first of two recommended doses [69,130]. It is, as such, indicated for post-exposure prophylaxis.

## 3.3 Active immunization

### *3.3.1 Pre-exposure prophylaxis*

The efficacy of formaldehyde-inactivated hepatitis A vaccines was demonstrated in two pivotal, double-blind randomized control trials conducted in Thailand [68] and the USA [69] in the early 1990s. In Thailand, HAVRIX® and a control hepatitis B vaccine were administered to 40 119 children aged between 1 and 16 years living in an area with high incidence of hepatitis A. HAVRIX® 360 EU/dose was administered intramuscularly at 0, 1 and 12 months. Protective efficacy against clinically and serologically confirmed hepatitis A disease was demonstrated in 94% and 99% of vaccinees after the second and third doses respectively (95% CI=79%–99%) [68]. Serologically confirmed hepatitis A through detection of anti-HAV(IgM) antibodies was documented in 38 episodes of illness in the control group, as compared to two cases in vaccinees. A similar, double-blind placebo-controlled trial was conducted in upstate New York, USA, in 1037 children aged 2–16 years, who received two doses of 25 units of VAQTA® or placebo at 0 and at 6–18 months. Vaccine efficacy of 100% was already established following the first dose [69] (95% CI=87.3%) [69].

Another smaller-scale randomized, placebo-controlled, double-blind trial of a single dose of EPAXAL®, a virosome formulated, aluminium-free inactivated HAV vaccine, was conducted in Nicaragua [71]. Two hundred and seventy-four children (age range 1.5–6 years) received vaccine or placebo injections, and 239 children, seronegative for hepatitis A, were included in the primary efficacy analysis. HAV infection was diagnosed through positive anti-HAV IgM in four children in the vaccine group and 22 children in the placebo group. The vaccine was 100% efficacious by week 6. For the entire study period, primary efficacy analysis was 84.6% (95% CI,54.7–96.1%) and 71.6% (95% CI, 40.5–87.6%) by intent to treat analysis.

All HAV vaccines manufactured in the western hemisphere are highly immunogenic and of comparable efficacy. HAV vaccines of different brand names are interchangeable as shown for HAVRIX®, AVAXIM®, EPAXAL® and VAQTA® [163–165].

In 2007, China introduced universal vaccination for 18-month-old toddlers. Half of the children received two intramuscular doses of formaldehyde-inactivated vaccines and half received one subcutaneous dose of a live attenuated vaccine. Follow-up evaluation revealed seroconversion rates of 83–91% in recipients of live attenuated vaccines. Protective efficacy was documented in 90–95% and 95–100% in recipients of live attenuated and formaldehyde-inactivated vaccines respectively [77].

### *3.3.2 Post-exposure prophylaxis*

Clinical trials of hepatitis A vaccines in the early 1990s provided unequivocal proof that pre-exposure prophylaxis of hepatitis A is effective and safe. Preliminary evidence obtained in a number of clinical trials suggested already that post-exposure immunization against hepatitis A may also have effectiveness similar to that of Ig, provided that immunization is started within two weeks of exposure.

Support for this assumption was initially obtained from the hepatitis A efficacy trial conducted in the USA where the VAQTA® vaccine was administered during an HAV outbreak and no new cases of acute hepatitis were identified from day 17 onwards after vaccination [69]. There was a similar experience in Slovakia where an outbreak of hepatitis A was interrupted by vaccination with HAVRIX® [166]. Later, a limited randomized controlled trial in Italy revealed a 79% protective efficacy of post-exposure immunization in household contacts of acute hepatitis A cases (95% CI=7%–95%) [167]. In Israel, as in Slovakia, prompt intervention with an active vaccine in a community outbreak of hepatitis A led to effective control of an epidemic within two weeks of starting the intervention, in contrast to the relatively poor performance of Ig [168]. Intervention in a major outbreak of hepatitis A in Ukraine led to a 41-fold drop in incidence of infection following a single-dose injection of AVAXIM® [169].

A pivotal support for establishing the effectiveness of post-exposure prophylaxis with an inactivated HAV vaccine has been reported from Kazakhstan [156]. In this controlled clinical trial, 1090 household and day-care contacts (aged 2–40 years) of index cases with acute hepatitis were randomized to receive hepatitis A vaccine or Ig. Transmission of HAV, confirmed by anti-HAV IgM, occurred in 4.4% and 3.3% of the study groups respectively (RR 1.35; 95% CI=0.70–2.67). Following a recent update by the United States Advisory Committee on Immunization Practices (ACIP), post-exposure immunization with an active HAV vaccine is now gaining accelerated momentum worldwide [170]. However, success of intervention in community outbreaks and epidemics through active vaccination requires a close public health surveillance and intervention system and, most importantly, compliance of the community where the intervention takes place. An example of failure of such an intervention occurred in the 1990s where first-dose coverage of vaccine was only 20–45% with little impact on herd immunity and incidence [171].

Altogether, the use of hepatitis A vaccines instead of Ig for post-exposure prophylaxis has a number of advantages, including induction of long-term protection against HAV, ease of administration and acceptance at a similar cost per dose [162].

# 4. Determinants of the immune response to immunization

## 4.1 Risk groups and risk factors

### *4.1.1 Age, gender, weight and smoking*

***Age:*** The physiology of the humoral, innate and cellular immune system in older age is often altered, leading to impaired immune responses [172,173]. This may have special implications for vaccination of older individuals who require protection against vaccine-preventable infections – i.e. international travellers from countries with low or very low endemicity for HAV to countries with intermediate and high endemicity [174–176]. This population is at risk of contracting HAV infection which is associated with increased morbidity, a higher proportion of hospitalization and higher mortality, as compared to younger persons below the age of 50 years [177]. Data on seroprotection rates in vaccinees older than 45 years are limited [174–178]; however, one uncontrolled study using EPAXAL® given in two doses, 12 months apart, revealed a blunted seroconversion rate of 65% after the first dose, compared to 100% in the younger control group. However, this difference in immunogenicity of the first dose disappeared after administration of the booster dose leading to 97% and 100% seroprotection rates respectively [179]. Similar results were obtained in other studies [174,175]. Thus, it has been suggested that HAV-susceptible individuals over the age of 45–50 years may require two vaccine doses, preferably four weeks apart, prior to departure on travel to endemic areas. However, a recent retrospective analysis does not support the introduction of such a strategy [180].

***Gender:*** As observed with other vaccines, anti-HAV antibody levels were reported to be higher in female vaccinees following a primary and booster injection, when compared to males [178]. However, in view of the excellent protective antibody levels induced by HAV vaccines, this difference in gender response has no practical consequences [178,179,181].

***Weight:*** Limited data suggest that better seroprotection rates after a first dose of 25U of VAQTA® were significantly associated with lower body weight and body mass index (BMI) [176]. A booster dose is expected to minimize the effect of overweight on the immunogenicity of HAV vaccines.

***Smoking:*** Results of a single study in HIV patients who received two or three doses of HAVRIX® 1440 EU at 0 and 24 weeks – or at 0, 4 and 24 weeks – revealed that absence of smoking was an independent predictor of response to vaccination [182].

### *4.1.2 Passively-acquired maternal anti-HAV antibodies*

The concentration of anti-HAV antibody levels in babies born to seropositive mothers who acquired HAV through natural infection remains high during the first six months postpartum but declines significantly by 12 months of age [183]. Newborns and young infants born to anti-HAV-positive mothers have a blunted humoral immune response to hepatitis A vaccination which may last for six months or more postpartum [183–187]. Although depression of the quantitative humoral immune response to vaccination may reach 30–90% compared to naïve newborns, this effect of passively-transferred maternal anti-HAV antibodies does not increase the risk for acquiring HAV infection. In such vaccinated babies, vaccine-induced anti-HAV IgG titres are still far above the required protective levels. Evidence that this effect is of negligible immunological significance is derived from the observation that such babies still develop an excellent anamnestic immune response to a booster vaccination given within 1–6 years of birth [183]. Furthermore, although paediatric HAV vaccines are licensed only for babies from 12 months of age, HAV immunization of 6–12-month-old children has been shown to be safe and sufficiently immunogenic even in the presence of passively transferred maternal antibodies [183–186].

### *4.1.3 Passively-acquired anti-HAV antibodies with immune globulin*

As also observed in newborns to seropositive mothers, immunogenicity of HAV vaccines is suppressed, although to a lesser extent, following concurrent passive and active immunization with Ig and vaccine respectively [158,159]. However, interference from passively-transferred anti-HAV antibodies with the humoral immune response to vaccination is of minor immunological consequence since it does not affect vaccine-induced protective immunity and response to booster vaccination is excellent. In an increasing number of countries worldwide passive Ig are no longer commercially available.

### *4.1.4 Immune suppression*

#### 4.1.4.1 Human immunodeficiency virus (HIV)

Patients infected with HIV types 1 or 2, including intravenous drug users, MSM and recipients of multiple blood transfusions (i.e. patients with haemophilia) are at increased risk for contracting HAV, HBV and HCV infection. A relatively high (5.8%) incidence of HAV has been reported in HIV patients in France, a country of low HAV endemicity [188]. Human immunodeficiency virus patients with HAV coinfection developed protracted hepatitis A viraemia with slow clinical resolution compared to non-HIV patients with a normal $CD_4$ cell count. Furthermore, seroconversion following vaccination may be blunted, leading to a decline in anti-HAV(IgG) antibodies [182,189–194]. However, vaccination against hepatitis A does not affect HIV viral load, can be given to patients who receive antiviral treatment, is safe and, in general, is almost as immunogenic in HIV-infected children and adults with a $CD_4$ count greater than 300/mm$^3$ and an HIV viral load lower than 1000 copies/mL, as in non-HIV subjects. Protection was achieved in up to 100% of children and in 69–83% of adults with HIV using the standard two-dose regimen at a 0 and 24-week interval between vaccine injections [193]. However, antibody levels achieved after the first and second vaccine dose may be lower compared to those in healthy subjects, and sometimes a third vaccine dose is warranted (especially in patients with a high HIV viral load and a $CD_4$ count below 300 cells/mm) preferably after suppression of viraemia and restitution of the $CD_4$ cell count [194].

(See the review of performance of HAV vaccines in the era of highly active antiretroviral therapy (HAART) [195].) Seroprotection should be confirmed using a conventional immunoassay for detection of anti-HAV(IgG) antibodies in such patients [182,194]. Data on long-term protection following HAV immunization in HIV patients are still lacking.

#### 4.1.4.2 Solid organ and stem-cell transplantation

In general, immune-suppressed patients who underwent organ transplantation have a blunted immune response to hepatitis A vaccines [196–199]. As shown in renal transplant patients, only 72% of 39 patients developed protective antibody levels [196]. Furthermore, kidney and liver transplant patients may lose their protection over time, as shown also in haematopoeitic stem-cell transplant patients, and especially in those with graft versus host disease [196–199].

### *4.1.5 Chronic liver disease*

The case fatality of HAV infection in patients with chronic liver disease is 23–58-fold higher compared to that is previously healthy infected adults, as was shown in two studies in patients with HCV and HBV [200,201]. Doubts have been raised regarding the cost-effectiveness of HAV immunization in patients with chronic HCV infection [50]. Yet immunization is a simple and safe procedure which can prevent hepatic decompensation. Immunization against HAV of children and adults with compensated chronic liver disease who do not receive immunosuppressive therapy generally leads to seroprotection rates similar to those of healthy subjects. However, anti-HAV(IgG) antibody levels are lower compared to those of healthy vaccines, and GMC levels are inversely proportional to the degree of liver failure [202–206]. In one study conducted in 89 seronegative children between the ages of 1 and 16 years, seroconversion rates following immunization with 720 EU of HAVRIX® reached 76% as compared to 94% in a control group with anti-HAV titres of 107 and 160 IU/mL respectively [206]. A booster dose given at month 6 led, however, to 97% and 100% seroconversion rates respectively, compensating for the slower response after the first dose. Overall, seroprotection rates following immunization are lower and decline faster in patients with decompensated liver disease. Criteria for vaccine booster doses have not been established in this population.

### *4.1.6 Co-administration of hepatitis A vaccines with other childhood or traveller vaccines*

Concurrent administration of a number of routine childhood and traveller monovalent and trivalent vaccines with an HAV vaccine does not lead to biologically significant interference in the immunogenicity, reactogenicity and safety of the individual vaccines. As demonstrated in a number of controlled studies conducted among 12–15-month-old infants, in children under 18 years and in adults, inactivated HAV vaccines can be administered simultaneously with vaccines for diphtheria, tetanus, acellular pertussis (DTaP), polio (oral and inactivated), Haemophilus influenza (Hib), MMR, typhoid (oral and intramuscular), hepatitis B, cholera, Japanese encephalitis, rabies and yellow fever [80,165,207–211]. It is recommended that injections should be given at different sites.

A number of combination vaccines have been developed that include hepatitis A and B (TWINRIX® and AMBIRIX®) [81–85,212] or hepatitis A and typhoid polysaccharide (HEPATRIX®, VIATIM® and VIVAXIM®) [32,137–140].

TWINRIX® contains 720EU of HAV antigen and 20 ug of HBsAg per dose, has been formulated with aluminium phosphate and aluminium hydroxide, and is given in three doses at 0, 1 and 6-month intervals in adults over 18 years of age. In Canada and some European countries, a half dose is available for younger ages. AMBIRIX® is a similar 2-dose combined hepatitis A and B vaccine distributed in Europe for 1–15-year-olds. Protective antibody levels against HAV and hepatitis B in recipients of the combination vaccine and the individual monovalent vaccines are comparable and long-lasting [144].

Combination vaccines against typhoid and hepatitis A are intended for adult travelers [213–216]. A combined vaccine VIATIM®)/VIVAXIM®) is given as a priming dose on day 0 followed by a booster dose of hepatitis A vaccine at month 6 and up to 3 years. Protective antibody levels against hepatitis A at 3 years are comparable to those obtained following immunization with the monovalent vaccines, with hypo-responsiveness reported for the typhoid vaccine [213].

## 4.2 Interchangeability of HAV vaccines

HAV vaccines are interchangeable. Crossover immunization from one HAV vaccine brand to another has been shown to be effective and safe in children and adults. Comparative immune reactivity following crossover immunization has been documented for VAQTA®, EPAXAL®, AVAXIM® and HAVRIX® [163,164,217,218], as well as for AVAXIM® and VAQTA® following combined immunization against HAV and typhoid [219].

## 4.3 Immunization strategies for protection against hepatitis A

Similar to experience previously gained with other vaccine-preventable infections, immunization strategies against hepatitis A have progressively changed over the past 20 years [29,55,70,127,220,221]. As more information has become available on the extraordinary immunogenicity, effectiveness and safety of hepatitis A vaccines, immunization strategies have shifted from the vaccination of persons belonging to specific risk groups to universal vaccination campaigns in specific regions within one country, and then to universal vaccination which is, however, still restricted to a limited number of countries [45,55,127,222–224] .

### 4.3.1 *Individual immunization of defined populations at risk*

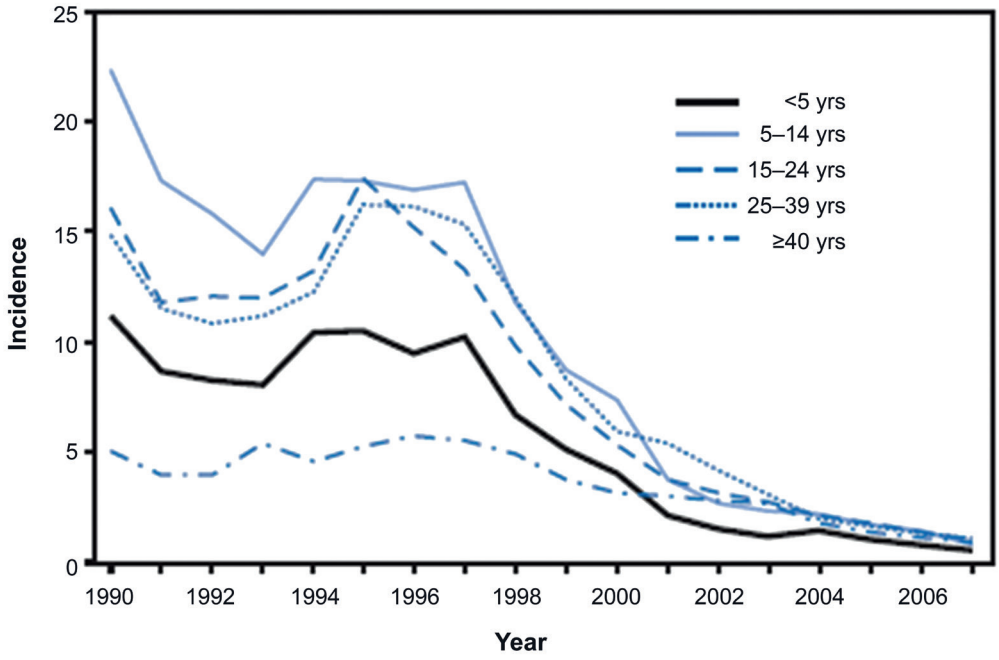
Early strategies for pre-exposure prophylaxis included immunization of defined risk groups for contracting hepatitis A (Table 1). These risk groups include international travellers to areas with intermediate and high endemicity of hepatitis A, MSM, people who inject drugs, patients with chronic liver disease, food-handlers, day-care centre staff, caretakers of nonhuman primates, parents of adoptees born in HAV-endemic countries, children of refugees and migrants from HAV-endemic countries, garbage and sewage workers, and patients with blood-clotting disorders receiving blood- derived products. (Homeless persons in the USA have recently been added to these risk groups) [225]. Although this policy has provided individual protection to vaccinees at risk, it has had little practical impact on the reduction of disease incidence/burden in low- and very low-endemicity settings, as demostrated by the massive hepatitis A outbreaks among MSM in some European Union countries.[d] A vast majority of National Immunization Technical Advisory Groups in Europe continue to recommend this risk group policy with variable uptake and limited efficacy. The recent outbreaks of hepatitis A in MSM, homeless persons and HIV populations in Asia, Europe and the USA provide examples of the limitations of uptake of the strategy [225–231].

### 4.3.2 *Regional vaccination of paediatric subpopulations at risk*

The effectiveness of universal vaccination projects in distinct regions within the same country targeting paediatric populations at higher risk of HAV infection (as compared to neighbouring regions) was demonstrated in a number of regions worldwide. Three demonstration projects conducted in the USA in native Americans in Alaska and in Butte County, California, led to a 94–97% reduction in incidence of reported symptomatic acute hepatitis A within 6–10 years, reaching an unprecedentedly low rate of 0.1 cases per 100 000 at a vaccine coverage of 50–80% in Alaska [224]. As a consequence of these successful projects, the United States ACIP in 1999 issued a recommendation to introduce universal hepatitis A vaccination into routine childhood vaccination (two doses in children over 2 years of age and catch-up at 10–12 years) in 17 states of the USA with an annual incidence of over 20 cases per 100 000. Follow-up surveillance revealed that, despite variable first vaccine coverage of 50–80%, a progressive decline in reported incidence of hepatitis A was observed from 21.1 cases per 100 000 to 2.5 cases per 100 000, representing an 88% drop (Figures 4a and 4b) [45,224]. Similar projects were introduced in Puglia, Italy, in 1997 [232], in Catalonia, Spain, in 1998 [233], and in North Queensland, Australia, in 1999 [234], leading to a 90–97% decline in the reported incidence in these regions.

[d] See: *https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/hepatitis-a-virus-EU-EEA-1975-2014.pdf*, accessed 15 April 2019.

**Figure 4a: Age-adjusted hepatitis A incidence (per 100,000 population) in the USA by age and year 1990–2007 following introduction of HAV vaccination**




*Reproduced, by permission of the publisher, from Daniels et al.*[45]
*See: https://www.cdc.gov/mmwr/preview/mmwrhtml /ss5803a1.htm, accessed 16 April 2019.*

The results of these highly successful vaccination projects in selected geographical regions and communities worldwide suggested that universal vaccination of children in communities at risk is effective and will lead to herd immunity even with moderate coverage. These early projects paved the way for the introduction of universal vaccination against hepatitis A in selected countries with intermediate endemicity in transition and defined patterns of HAV transmission.

### *4.3.3 Universal vaccination of toddlers*

Universal vaccination of babies born in countries of intermediate endemicity of HAV or in areas in transition from high to intermediate endemicity is being gradually introduced in selected regions worldwide.

In the 1990s, Israel, with a population of some 6 million people, was in transition from high to intermediate endemicity. The overall incidence of HAV infection revealed seasonal fluctuations between 33 and 70 cases per 100 000 during 1992–1998, reaching 120 cases per 100 000 in children aged 5–9 years. The decision to introduce universal vaccination to toddlers, irrespective of regional differences in incidence, was based on cost–benefit analysis and long-term surveillance data, suggesting that children were the main source for transmission of HAV in babies and adults alike. The programme, which started in 1999, offers free-of-charge vaccine (HAVRIX® 720 El.U) to 18-month-old babies, with a booster dose at the age of 24 months. At a vaccination coverage of 90% and 85% for the first and second doses respectively, the annual incidence of hepatitis A

dropped sharply within 2–3 years of programme initiation, reaching an overall decline of 95% compared to pre-vaccination rates [222]. Thus, immunization of some 3% of the overall population annually led to a marked decrease in attack rates of HAV infection in all age groups and a shift from a state of HAV intermediate endemicity to very low endemicity with an annual incidence of around 2.5 cases per 100 000 (Figure 5, Figure 6, Table 3). However, despite the successful increase in herd immunity following universal mass vaccination, which has now been documented for more than 15 years (Figure 6), and the control of HAV infection achieved in Israel, occasional outbreaks still occur. Such an outbreak was recorded in 2012–2013 in Tel-Aviv and was associated with the detection of HAV in sewage samples and confirmed by phylogenetic analysis [235]. This occurrence was apparently facilitated, at least in part, through contact between non-immunized susceptible populations and migrant workers from countries or regions that are endemic for HAV [236].

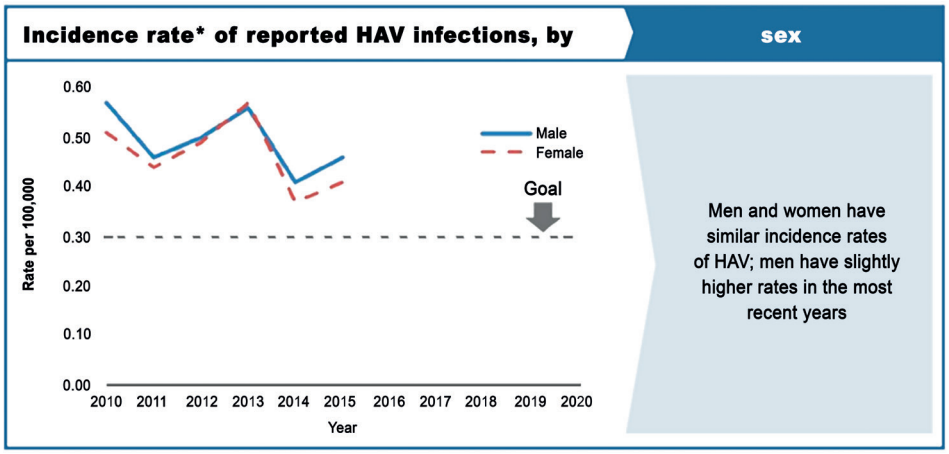
**Table 3. Mean age-specific incidence per 100 000 obtained by passive surveillance before and following universal vaccination against HAV in toddlers in Israel. Vaccination started in 1995**

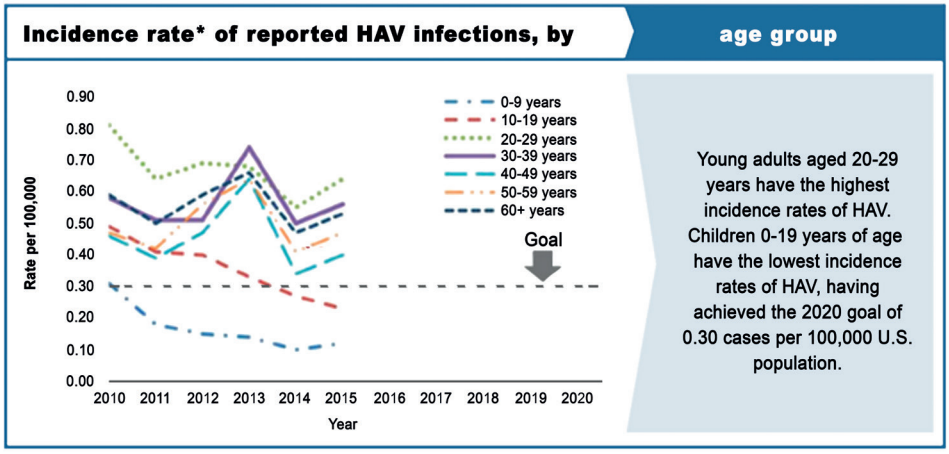
| Jewish population | | | | | | |
|---|---|---|---|---|---|---|
| **Age (years)** | **<1** | **1–4** | **5–9** | **10–14** | **15–44** | **45–64** |
| **1993–1998** | 19.1 | 105.7 | 181.2 | 85.5 | 30.1 | 6.8 |
| **2002–2004** | 4.2 | 0.5 | 1.2 | 2.2 | 2.1 | 0.7 |
| **Incidence reduction (%)** | 78.1 | 99.5 | 99.3 | 97.4 | 92.9 | 89.6 |
| **Non-Jewish population** | | | | | | |
| **Age (years)** | **<1** | **1–4** | **5–9** | **10–14** | **15–44** | **45–64** |
| **1993–1998** | 34.2 | 186.8 | 224.6 | 73.7 | 7 | 2.7 |
| **2002–2004** | 2.6 | 5.9 | 18.4 | 8.4 | 2.2 | 0.2 |
| **Incidence reduction (%)** | 92.3 | 96.8 | 91.8 | 88.6 | 67.9 | 93.9 |

*Reproduced, by permission of the publisher, from Dagan et al. [222]*

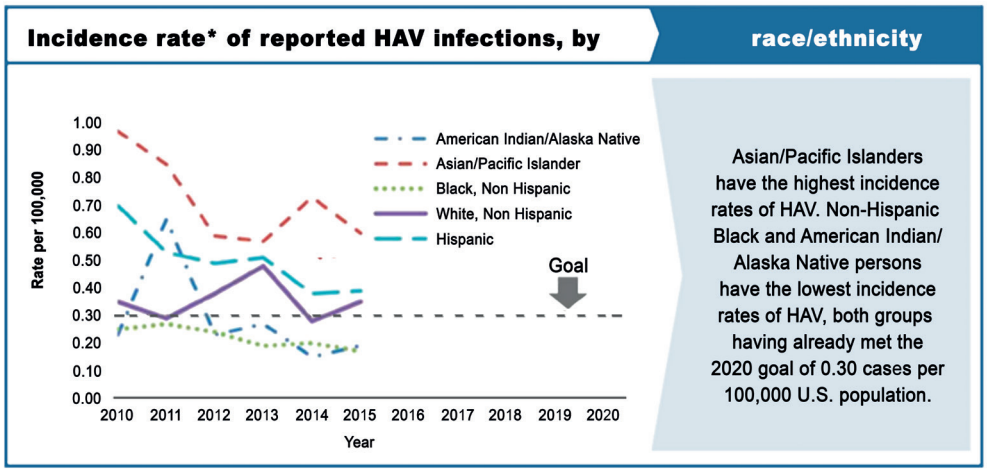
Dagan R, Leventhal A, Anis E, Slater P, Ashur Y, Shouval D. Incidence of hepatitis A in Israel following universal immunization of toddlers. Jama. 2005;294:202–10.

## Figure 4b: Follow-up on incidence of HAV infection in the USA



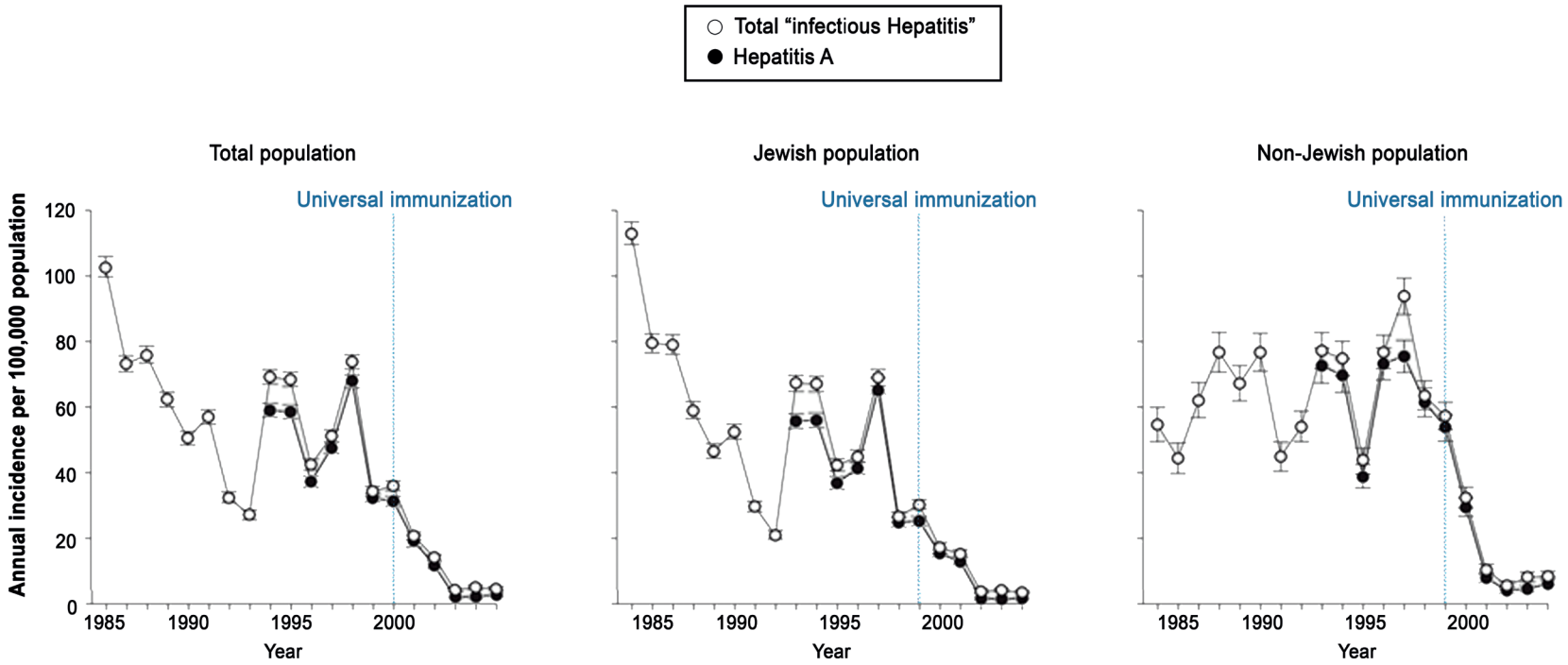

*See: https://www.cdc.gov/hepatitis/policy/pdfs/NationalProgressReport.pdf, accessed November 2017.*




* *Rate per 100 000 United States population*

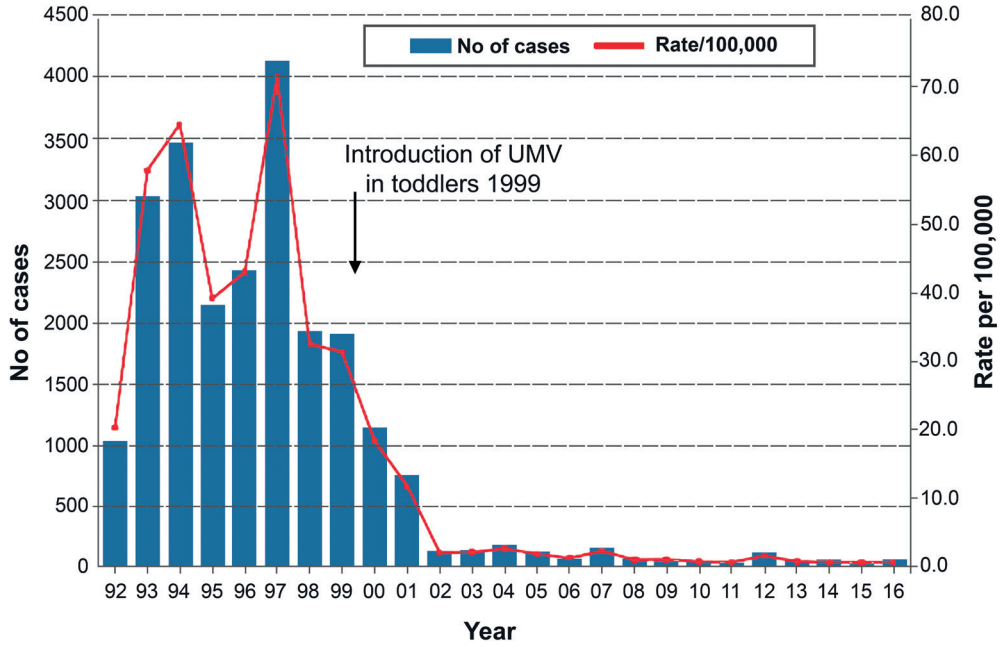
*Data reproduced by permission of the United States Centers for Disease Control and Prevention data on progress of viral hepatitis elimina tion UnitedStates – National Report 2017*

## Figure 5: Annual incidence rates in Israel of reported infectious hepatitis (A, B, C and non-specified), 1993–2004, and hepatitis A only, 1985–2004




*Reproduced, by permission of Elsevier, from Dagan et al.*[222]

**Figure 6: Incidence of acute hepatitis A in Israel, January 1992–December 2016. Universal mass vaccination was started in 1999 using an inactivated HAV vaccine at 18 and 24 months of age. Data collected through passive surveillance by the Israel Ministry of Health.**




*Reproduced by permission from Lemon et al. [6]*

WHO recommends integration of universal vaccination against HAV in national immunization schedules for children aged 1 year and above if justified on the basis of acute HAV incidence, declining endemicity from high to intermediate and cost-effectiveness [1]. A recent survey analysed the impact of universal mass vaccination on incidence of hepatitis A in selected regions worldwide, using two doses of monovalent inactivated hepatitis A vaccines [237]. Data collected from a total of 27 studies conducted in Argentina, China, Greece, Israel, Panama, the United States and Uruguay, revealed a marked decline in the incidence of hepatitis A after introduction of universal vaccination in all surveyed countries (except for Greece where the declining baseline incidence of HAV infection prior to vaccination had a statistical impact on the results). Declines in incidence were observed, irrespective of age at first vaccine dose (12–24 months), vaccine brand or vaccine coverage. The incidence in nonvaccinated age groups also decreased, suggesting herd immunity but also rising susceptibility. Long-term anti-HAV antibody persistence was documented up to 20 years after a 2-dose immunization schedule given 6–12 months apart [144]. A similar short- and intermediate-term impact was observed in Argentina and China using a single-dose immunization schedule with inactivated or live attenuated vaccines respectively. In addition to a marked reduction in the incidence of HAV infection, the introduction of universal mass vaccination in selected countries and regions has had additional indirect effects, including a reduction in age-adjusted mortality, fulminant hepatitis and referral for liver transplantation in Argentina [238], a decline in hospitalization rates for severe hepatitis A in Greece [237] and a drop in outbreaks of hepatitis A in day-care centres in Israel [236].

### 4.3.4 *Single-dose immunization*

Cumulative experience using hepatitis A vaccines, irrespective of the manufacturer, has confirmed the unprecedented high immunogenicity of these vaccines, as manifested by anti-HAV(IgG) seroconversion rates in 88% of vaccinees within two weeks of the priming dose and rising to 97–100% at weeks 4–6 [34,130,239]. Furthermore, results from clinical trials in the USA, and intervention during HAV outbreaks in several other countries, suggest that a single HAV dose may be sufficient to prime the immune response and interrupt the spread in communities at risk [69,71,168]. Moreover, international travellers who received one priming dose of an HAV vaccine, either as a monovalent vaccine or in combination with a typhoid vaccine, and had a booster dose given 4–8 years later, developed a robust anamnestic response against HAV [240,241]. In 2005, public health authorities in Argentina began a universal immunization programme in 12-month-old babies [146]. Because of economic constraints, a decision was made to use a single-dose vaccine schedule. This decision was based on a prediction that an encounter of vaccinees with wild-type HAV after a single dose would result in an anamnestic immune response. The original incidence of reported HAV in Argentina fluctuated between 70.5 and 173.8 cases per 100 000 between 1995 and 2004. So far the results of this strategy have been excellent. At a vaccination coverage of 95% in 2006, incidence of symptomatic viral hepatitis A dropped sharply in 2007 to around 10 cases per 100 000 in all age groups, representing a more than 80% decrease in incidence. The experience gained in Argentina, using a single vaccine dose strategy, is gaining momentum worldwide [145,242]. Consequently, the short- and intermediate-term effectiveness of such a policy, using a formaldehyde-inactivated or live attenuated vaccine has been documented in Nicaragua [243], Korea [244], China [245,246] and India [247], including persistence of vaccine-acquired immune memory against HAV [145]. It remains to be seen, however, whether a single-dose immunization policy will indeed provide long-term protection against HAV or whether a booster dose will after all be required, as predicted by some [248]. In view of the high immunogenicity of HAV vaccines, and given the rapid impact already observed in Argentina, the probability for acquiring natural infection in a immunized population, even with a single dose, is quite low.

### 4.3.5 *Hepatitis A outbreaks and vaccine supply*

Between June 2016 and July 2017, 16 European countries reported 1500 cases of confirmed acute HAV infection and 2660 cases of probable or suspected infection. Outbreaks of hepatitis A in MSM have been documented in several countries both within and outside Europe [227,229,231,249,250].[e] An outbreak also occurred among homeless persons in the USA [225]. In parallel, between 2016 and 2017, a shortage in supply of inactivated HAV vaccine occurred in Europe and the USA, apparently as a result of manufacturing issues and increase in demand. Consequently, public health authorities in the European Union and the USA had to redefine priorities regarding indication for pre- and post-exposure prophylaxis. Several options were considered and some implemented, including pre-vaccination screening for anti-HAV(IgG) in MSM and homeless persons, use of a paediatric HAV formulation for immunization of adults, vaccination with a sing le-dose strategy, and redefining the risk for travellers to previously-reported endemic HAV regions with improving sanitary and socioeconomic conditions [251]. The follow-up to the impact of these measures is pending.

[e] See: *http://invs.santepubliquefrance.fr/Dossiers-thematiques/Maladies-infectieuses/Hepatites-virales/Hepatite-A/Points-d-actualite/Epidemie-d-hepatite-A-en-France-et-en-Europe-Point-de-situation-au-27-juillet-2017*, accessed 15 April 2019.

# 5. Safety of hepatitis A vaccines

On the basis of the cumulative experience gained from using hundreds of million doses over the past 20 years, the overall safety profile of all formaldehyde-inactivated hepatitis A vaccines administered to children and adults has been excellent, irrespective of manufacturer [55,70,187,220,252–257]. The following information is quoted from a review by the United States Centers for Disease Control and Prevention (CDC) [55]:

> ***Reactogenicity:*** Local reactions, including soreness or tenderness at injection site, were reported in pre-licensure clinical trials in 56% (N=50 000) and 53% (N=10 000) of adult recipients of HAVRIX® and VAQTA® respectively, while in children these figures were at a range of 15% and 17%. Headaches were reported in 14% to 16% of adults for both vaccines respectively, and in 4% of children receiving HAVRIX® in whom ~8% had feeding problems.
>
> Mild injection site reactions have been reported with all inactivated hepatitis A vaccines. Symptoms are transient, more commonly reported in adults, and usually occur following the first dose. The rate of injection site reactions varies depending on the specific study. The most frequently reported local adverse events have included injection site soreness (14–27% in children, up to 43–56% in adults) and induration at the injection site (4%) with lower rates of injection site erythema and pain noted after booster doses in 9% of children and up to 24% of adults.
>
> ***Systemic adverse events:*** Mild systemic adverse events have been reported after all inactivated hepatitis A vaccines and, like local reactions, may be less frequent after booster doses. Symptoms have included headache (4% in children, up to 16% in adults), malaise (7%), anorexia, feeding problems (8%) and fatigue, fever, diarrhoea and vomiting occurring in less than 5% of vaccine recipients. Symptoms usually resolved within 48 hours.[f]

[f] See: WHO Information sheet. Observed rate of vaccine reactions. Hepatitis A vaccine. *https://www.who.int/vaccine_safety/initiative/tools/Hep_A_Vaccine_rates_information_sheet.pdf?ua=1*, accessed 15 April 2019.

***Serious Adverse Events (SAE):*** An estimated 1.3 million persons in Asia and Europe were vaccinated with HAVRIX® before the vaccine's licensure in the United States in 1995. Reports of serious adverse events received by the vaccine manufacturer, included anaphylaxis, Guillain-Barré syndrome, brachial plexus neuropathy, transverse myelitis, multiple sclerosis, encephalopathy and erythema multiform. The majority of these events occurred among adults, and approximately one third occurred among persons concurrently receiving other vaccines. For serious adverse events for which background incidence data can be estimated (e.g. Guillain-Barré syndrome and brachial plexus neuropathy), rates for vaccine recipients were no higher than would be expected for an unvaccinated population.

No vaccine-related serious adverse events were reported for approximately 40 000 children who were administered the 360EL.U. dose of HAVRIX® in the protective efficacy study (26). In a post-licensure study of 11 417 children and 25 023 adults who were administered VAQTA®, no serious adverse events occurred that were considered to be associated with administration of vaccine. A published post-licensure evaluation of safety among 2000 child and adult recipients identified no serious adverse events associated with VAQTA® [257].

According to information received from the China Centers for Disease Control, between 1992–2007, 60 million doses of mainly live attenuated HAV vaccines were administered in China. Since 2005, 8 million doses are distributed annually to 18-month-old toddlers and older children. With respect to the Chinese inactivated and live attenuated hepatitis A vaccines, experience during clinical trials and through passive surveillance did not identify any substantial safety issues [77]. It will be however essential to conduct rigorous high-quality postmarketing surveillance in selected communities to measure and monitor safety and adverse reactions. Studies of children vaccinated with live vaccines have shown shedding of the vaccine virus and possibly secondary, albeit "silent" infection among contacts. Thus, post-marketing surveillance is essential and may provide a context in which to conduct specific studies to examine the outcome of secondary infection and virus circulation if it occurs. In view of the volume of use of live hepatitis A vaccines in China and their potential usefulness in other countries, carefully collected and validated data on safety and efficacy will be valuable. Data of particular interest will be follow-up of molecular markers of attenuation, the genetic stability of attenuation markers after human passage, as well as further follow-up and information on clinical safety and efficacy to be demonstrated in well-conducted and sufficiently large clinical trials.[g]

[g] *http://www.who.int/wer/2010/wer8530/en/index.html*, accessed 15 April 2019.

## 5.1 Monovalent hepatitis A vaccine

No severe adverse events have been causally linked to hepatitis A vaccines. In pre-licensure clinical studies involving more than 60 000 persons vaccinated, no serious adverse reactions were definitively attributed to hepatitis A vaccine. Post-licensure AEFI reports after vaccination of an estimated 1.3 million persons included anaphylaxis, Guillain-Barré syndrome, brachial plexus neuropathy, transverse myelitis, multiple sclerosis, and erythema multiforme but none of those events could be conclusively linked to vaccine administration. Most of these events occurred among adults, and approximately one third have occurred among persons concurrently receiving other vaccines. For adverse events for which background incidence data are available (e.g. Guillain-Barré syndrome and brachial plexus neuropathy) the rates among vaccine recipients are not higher than would be expected for an unvaccinated population.

# 6. Conclusions

After almost two decades since hepatitis A vaccines first became available, it can be stated without reservation that these vaccines are among the most immunogenic, safe and well-tolerated vaccines ever produced. Two doses of a hepatitis A vaccine will generate a long-lasting protective cellular and humoral immune response in children and adults which is expected to last for decades, and possibly for a lifetime in immune-competent recipients. Short-term and intermediate-term protective immunity against HAV has also been documented following injection of a single vaccine dose. In view of the many countries which are currently in transition from high to intermediate endemicity, it can be expected that large cohorts of young adults and children worldwide will remain susceptible to HAV infection transmitted via unsafe food products, travel or person-to-person contact.[h] Public health agencies in such countries will have to strengthen surveillance and reassess the need for universal vaccination depending, among other factors, on the disease burden, available resources and health priorities. Meanwhile, duration of immune memory against HAV in recipients of HAV vaccines should be followed with special emphasis on vaccinees who received a single-dose injection (using live attenuated or inactivated HAV vaccines) and those who are immunocompromised and at risk.

[h] See: *https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/hepatitis-a-virus-EU-EEA-1975-2014.pdf*, accessed 16 April 2019.

# 7. References


1. WHO position paper on hepatitis A vaccines, June 2012. *Wkly Epidemiol Rec. 2012;87:261–76.*
2. **Feinstone SM.** History of the discovery of hepatitis A virus. In: Lemon SM, Walker CM, editors. Enteric hepatitis viruses. *Cold Spring Harb Perspect Med. 2018:9–22.*
3. **Teo CG.** 19th-century and early 20th-century jaundice outbreaks, the USA. *Epidemiol Infect. 2018;146:138–46.*
4. **MacCallum FO.** 1971 International Symposium on Viral Hepatitis. Historical perspectives. *Can Med Assoc J. 1972;106:Suppl:423–6.*
5. **Feinstone SM, Kapikian AZ, Purcell RH.** Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. *Science. 1973;182:1026–8.*
6. **Nainan OV, Xia G, Vaughan G, Margolis HS.** Diagnosis of hepatitis A virus infection: a molecular approach. *Clin Microbiol Rev. 2006;19:63–79.*
7. **Martin A, Lemon SM.** Hepatitis A virus: from discovery to vaccines. *Hepatology. 2006;43:S164–72.*
8. **Vaughan G, Goncalves Rossi LM, Forbi JC, de Paula VS, Purdy MA, Xia G et al.** Hepatitis A virus: host interactions, molecular epidemiology and evolution. *Infect Genet Evol. 2014;21:227–43.*
9. **Lemon SM, Ott JJ, Van Damme P, Shouval D.** Type A viral hepatitis: a summary and update on the molecular virology, epidemiology, pathogenesis and prevention. *J Hepatol. 2018;68:167–84.*
10. **Kaplan G, Totsuka A, Thompson P, Akatsuka T, Moritsugu Y, Feinstone SM.** Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus. *EMBO J. 1996;15:4282–96.*
11. **Wang X, Ren J, Gao Q, Hu Z, Sun Y, Li X et al.** Hepatitis A virus and the origins of picornaviruses. *Nature. 2015;517:85–8.*
12. **Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L et al.** A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. *Nature. 2013;496:367–71.*
13. **Siegl G, Weitz M, Kronauer G.** Stability of hepatitis A virus. *Intervirology. 1984;22:218–26.*
14. **Reid TM, Robinson HG.** Frozen raspberries and hepatitis A. *Epidemiol Infect. 1987;98:109–12.*

15. **Sattar SA, Jason T, Bidawid S, Farber J.** Foodborne spread of hepatitis A: Recent studies on virus survival, transfer and inactivation. *Can J Infect Dis. 2000;11:159–63.*

16. **McCaustland KA, Bond WW, Bradley DW, Ebert JW, Maynard JE.** Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol. 1982;16:957–8.*

17. **Asher LV, Binn LN, Mensing TL, Marchwicki RH, Vassell RA, Young GD.** Pathogenesis of hepatitis A in orally inoculated owl monkeys (Aotus trivirgatus). *J Med Virol. 1995;47:260–8.*

18. **Dotzauer A, Gebhardt U, Bieback K, Göttke U, Kracke A, Mages J et al.** Hepatitis A virus-specific immunoglobulin A mediates infection of hepatocytes with hepatitis A virus via the asialoglycoprotein receptor. *J Virol. 2000;74:10950–7.*

19. **Feigelstock D, Thompson P, Mattoo P, Zhang Y, Kaplan GG.** The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. *J Virol. 1998;72:6621–8.*

20. **Das A, Hirai-Yuki A, Gonzalez-Lopez O, Rhein B, Moller-Tank S, Brouillette R et al.** TIM1 (HAVCR1) is not essential for cellular entry of either quasi-enveloped or naked hepatitis A virions. *MBio. 2017;8.*

21. **Jacobsen KH.** Globalization and the changing epidemiology of hepatitis A virus. *Cold Spring Harb Perspect Med. 2018;8.*

22. **Jacobsen KH, Koopman JS.** Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect. 2004;132:1005–22.*

23. **Jacobsen KH, Wiersma ST.** Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine. 2010;28:6653–7.*

24. **Gripenberg M, Aloysia D'Cor N, L'Azou M, Marsh G, Druelles S, Nealon J.** Changing sero-epidemiology of hepatitis A in Asia Pacific countries: a systematic review. *Int J Infect Dis. 2018;68:13–7.*

25. **Carrillo-Santisteve P, Tavoschi L, Severi E, Bonfigli S, Edelstein M, Byström E et al.** Seroprevalence and susceptibility to hepatitis A in the European Union and European Economic Area: a systematic review. *Lancet Infect Dis. 2017;17:e306–e19.*

26. **Armstrong GL, Bell BP.** Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. P*ediatrics. 2002;109:839–45.*

27. **FitzSimons D, Hendrickx G, Vorsters A, Van Damme P.** Hepatitis A and E: update on prevention and epidemiology. *Vaccine. 2010;28:583–8.*

28. **Van Damme P, Van Herck K.** A review of the long-term protection after hepatitis A and B vaccination. *Travel Med Infect Dis. 2007;5:79–84.*

29. **Hendrickx G, Van Herck K, Vorsters A, Wiersma S, Shapiro C, Andrus JK et al.** Has the time come to control hepatitis A globally? Matching prevention to the changing epidemiology. *J Viral Hepat. 2008;15(Suppl 2):1–15.*

30. Canada communicable disease report / Relève des maladies transmissibles au Canada. 2000;26:33–8.

31. **Havelaar AH, Kirk MD, Torgerson PR, Gibb HJ, Hald T, Lake RJ et al.** World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS Med. 2015;12:e1001923.*

32. **Gust ID.** Epidemiological patterns of hepatitis A in different parts of the world. *Vaccine. 1992;10(Suppl 1):S56–8.*

33. **Aggarwal R, Goel A.** Hepatitis A: epidemiology in resource-poor countries. *Curr Opin Infect Dis. 2015;28:488–96.*

34. **Ashur Y, Adler R, Rowe M, Shouval D.** Comparison of immunogenicity of two hepatitis A vaccines – VAQTA and HAVRIX – in young adults. *Vaccine. 1999;17:2290–6.*

35. **Cooksley WG.** What did we learn from the Shanghai hepatitis A epidemic? *J Viral Hepat. 2000;7(Suppl 1):1–3.*

36. **Halliday ML, Kang LY, Zhou TK, Hu MD, Pan QC, Fu TY et al.** An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis 1991;164:852–9.*

37. **Kim YJ, Lee HS.** Increasing incidence of hepatitis A in Korean adults. *Intervirology. 2010;53:10–4.*

38. **Kim JD, Choi JY, Park CH, Song MJ, Jang JW, Bae SH et al.** [Clinical features of patients with fulminant hepatitis A requiring emergency liver transplantation: comparison with acute liver failure due to other causes]. *Korean J Hepatol;16:19–28.*

39. **Yoon EL, Sinn DH, Lee HW, Kim JH.** Current status and strategies for the control of viral hepatitis A in Korea. *Clin Mol Hepatol. 2017;23:196–204.*

40. **Centeno MA, Bes DF, Sasbon JS.** Mortality risk factors of a pediatric population with fulminant hepatic failure undergoing orthotopic liver transplantation in a pediatric intensive care unit. *Pediatr Crit Care Med. 2002;3:227–33.*

41. **Munne MS, Vladimirsky S, Moreiro R, Ciocca M, Cuarterolo M, Otegui L et al.** Molecular characterization of hepatitis A virus in children with fulminant hepatic failure in Argentina. *Liver Int. 2008;28:47–53.*

42. **Santos DC, Martinho JM, Pacheco-Moreira LF, Carvalho Viana de Araújo II C, Euzebio Pereira Dias de Oliveira BC, Vieira Lago B et al.** Fulminant hepatitis failure in adults and children from a Public Hospital in Rio de Janeiro, Brazil. *Braz J Infect Dis. 2009;13:323–9.*

43. **Bruni R, Taffon S, Equestre M, Chionne P, Madonna E, Rizzo C et al.** Key role of sequencing to trace hepatitis A viruses circulating in Italy during a large multi-country European foodborne outbreak in 2013. *PLoS One. 2016;11:e0149642.*

44. **Petrignani M, Verhoef L, Vennema H, van Hunen R, Baas D, van Steenbergen JE et al.** Underdiagnosis of foodborne hepatitis A, The Netherlands, 2008–2010 (1). *Emerg Infect Dis. 2014;20:596–602.*

45. **Daniels D, Grytdal S, Wasley A.** Surveillance for acute viral hepatitis – United States, 2007. *MMWR Surveill Summ. 2009;58:1–27.*

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

47. **Sjogren MH, Tanno H, Fay O, Sileoni S, Cohen BD, Burke DS et al.** Hepatitis A virus in stool during clinical relapse. *Ann Intern Med. 1987;106:221–6.*

48. **de la Rubia L, del Pozo F, Carrasco M, Oria de Rueda O, Cebrero M, Garcia de Frias E.** Persistence of anti-HAV IgM antibodies and relapse of hepatitis type A in childhood. *J Hepatol. 1993;17:421–2.*

49. **Rowe IA, Parker R, Armstrong MJ, Houlihan DD, Mutimer DJ.** Hepatitis A virus vaccination in persons with hepatitis C virus infection: consequences of quality measure implementation. *Hepatology. 2012;56:501–6.*

50. **Shouval D.** Hepatitis: new doubts about preventing HAV superinfection in chronic HCV. *Nat Rev Gastroenterol Hepatol. 2012;9:367–8.*

51. **Chen CM, Chen SC, Yang HY, Yang ST, Wang CM.** Hospitalization and mortality due to hepatitis A in Taiwan: a 15-year nationwide cohort study. *J Viral Hepat. 2016;23:940–5.*

52. **Murphy TV, Denniston MM, Hill HA, McDonald M, Klevens MR, Elam-Evans LD et al.** Progress toward eliminating hepatitis A disease in the United States. *MMWR Suppl 2016;65:29–41.*

53. **Krugman S, Giles JP.** Viral hepatitis. New light on an old disease. *JAMA 1970;212:1019–29.*

54. **Lednar WM, Lemon SM, Kirkpatrick JW, Redfield RR, Fields ML, Kelley PW.** Frequency of illness associated with epidemic hepatitis A virus infections in adults. *Am J Epidemiol. 1985;122:226–33.*

55. **Fiore AE, Wasley A, Bell BP.** Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep 2006;55:1–23.*

56. **Tong MJ, el-Farra NS, Grew MI.** Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. *J Infect Dis. 1995;171 (Suppl 1):S15–8.*

57. **Schiff ER.** Atypical clinical manifestations of hepatitis A. *Vaccine. 1992;10 (Suppl 1):S18–20.*

58. **Willner IR, Uhl MD, Howard SC, Williams EQ, Riely CA, Waters B.** Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. *Ann Intern Med. 1998;128:111–4.*

59. **Crowcroft NS, Walsh B, Davison KL, Gungabissoon U/ PHLS.** Advisory Committee on Vaccination and Immunisation. Guidelines for the control of hepatitis A virus infection. *Communicable disease and public health / PHLS 2001;4(3):213–27.*

60. **Taylor RM, Davern T, Munoz S, Han SH, McGuire B, Larson AM et al.** Fulminant hepatitis A virus infection in the United States: incidence, prognosis, and outcomes. *Hepatology. 2006;44:1589–97.*

61. **Kim JM, Lee YS, Lee JH, Kim W, Lim KS.** [Clinical outcomes and predictive factors of spontaneous survival in patients with fulminant hepatitis A]. *Korean J Hepatol. 2008;14:474–82.*

62. **Kim HY, Eyheramonho MB, Pichavant M, Gonzalez Cambaceres C, Matangkasombut P, Cervio G et al.** A polymorphism in TIM1 is associated with susceptibility to severe hepatitis A virus infection in humans. *J Clin Invest. 2011;121:1111–8.*

63. **Ciocca M.** Clinical course and consequences of hepatitis A infection. *Vaccine. 2000;18(Suppl 1):S71–4.*

64. **Ciocca M, Moreira-Silva SF, Alegria S, Galoppo MC, Ruttiman R, Porta G et al.** Hepatitis A as an etiologic agent of acute liver failure in Latin America. *Pediatr Infect Dis J. 2007;26:711–5.*

65. **Ferreira CT, Vieira SM, Kieling CO, Silveira TR.** Hepatitis A acute liver failure: follow-up of paediatric patients in southern Brazil. *J Viral Hepat. 2008;15(Suppl 2):66–8.*

66. **Vizzotti C, Gonzalez J, Rearte A, Urueña A, Pérez Carrega M, Calli R et al.** Single-dose universal hepatitis A immunization in Argentina: low viral circulation and high persistence of protective antibodies up to 4 years. *J Pediatric Infect Dis Soc. 2015;4:e62–7.*

67. **Provost PJ, Hilleman MR.** Propagation of human hepatitis A virus in cell culture in vitro. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1979;160:213–21.*

68. **Innis BL, Snitbhan R, Kunasol P, Laorakpongse T, Poopatanakool W, Kozik CA et al.** Protection against hepatitis A by an inactivated vaccine. *JAMA. 1994;271:1328–34.*

69. **Werzberger A, Mensch B, Kuter B, Brown L, Lewis J, Sitrin R et al.** A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med. 1992;327:453–7.*

70. **Vidor E, Dumas R, Porteret V, Bailleux F, Veitch K.** Aventis Pasteur vaccines containing inactivated hepatitis A virus: a compilation of immunogenicity data. *Eur J Clin Microbiol Infect Dis. 2004;23:300–9.*

71. **Mayorga Perez O, Herzog C, Zellmeyer M, Loaisiga A, Frosner G, Egger M.** Efficacy of virosome hepatitis A vaccine in young children in Nicaragua: randomized placebo-controlled trial. *J Infect Dis. 2003;188:671–7.*

72. **Jiang WP, Chen JT, Wang X, Wang YL , Liu Y , Chen WY et al.** Immunogenicity and safety of three consecutive lots of a new preservative-free inactivated hepatitis A vaccine (Healive): a double-blind, randomized and controlled trial. *Vaccine. 2008;26:2297–301.*

73. **Mao JS, Dong DX, Zhang HY, Chen NL, Zhang XY, Huang HY et al.** Primary study of attenuated live hepatitis A vaccine (H2 strain) in humans. *J Infect Dis. 1989;159:621–4.*

74. **Mao JS, Chai SA, Xie RY, Chen NL, Jiang Q, Zhu XZ et al.** Further evaluation of the safety and protective efficacy of live attenuated hepatitis A vaccine (H2-strain) in humans. *Vaccine. 1997;15:944–7.*

75. **Zhao YL, Meng ZD, Xu ZY, Guo JJ, Chai SA, Duo CG et al.** H2 strain attenuated live hepatitis A vaccines:protective efficacy in a hepatitis A outbreak. *World J Gastroenterol. 2000;6:829–32.*

76. **Wang XY, Xu ZY, Ma JC, Ghosh A, Sankaranarayanan VS, Aggarwal A et al.** Long-term immunogenicity after single and booster dose of a live attenuated hepatitis A vaccine: results from 8-year follow-up. *Vaccine. 2007;25:446–9.*

77. **Cui F, Liang X, Wang F, Zheng H, Hutin YJ, Yang W.** Development, production, and postmarketing surveillance of hepatitis A vaccines in China. *J Epidemiol. 2014;24:169–77.*

78. **Faridi MM, Shah N, Ghosh TK, Arankalle V, Aggarwal A, Sathiyasekaran M et al.** Immunogenicity and safety of live attenuated hepatitis A vaccine: a multicentric study. *Indian Pediatr. 2009;46:29–34.*

79. **Shen YG, Gu XJ, Zhou JH.** Protective effect of inactivated hepatitis A vaccine against the outbreak of hepatitis A in an open rural community. *World J Gastroenterol. 2008;14:2771–5.*

80. **Ambrosch F, Andre FE, Delem A, D'Hondt E, Jonas S, Kunz C et al.** Simultaneous vaccination against hepatitis A and B: results of a controlled study. *Vaccine. 1992;10(Suppl 1):S142–5.*

81. **Van Damme P, Van Herck K.** A review of the efficacy, immunogenicity and tolerability of a combined hepatitis A and B vaccine. *Expert Rev Vaccines. 2004;3:249–67.*

82. **Czeschinski PA, Binding N, Witting U.** Hepatitis A and hepatitis B vaccinations: immunogenicity of combined vaccine and of simultaneously or separately applied single vaccines. *Vaccine. 2000;18:1074–80.*

83. **Joines RW, Blatter M, Abraham B, Xie F, De Clercq N, Baine Y et al.** A prospective, randomized, comparative US trial of a combination hepatitis A and B vaccine (Twinrix) with corresponding monovalent vaccines (Havrix and Engerix-B) in adults. *Vaccine. 2001;19:4710–9.*

84. **Van Damme P, Van der Wielen M.** Combining hepatitis A and B vaccination in children and adolescents. *Vaccine. 2001;19:2407–12.*

85. **Knoll A, Hottentrager B, Kainz J, Bretschneider B, Jilg W.** Immunogenicity of a combined hepatitis A and B vaccine in healthy young adults. *Vaccine. 2000;18:2029–32.*

86. **Beeching NJ, Clarke PD, Kitchin NR, Pirmohamed J, Veitch K, Weber F.** Comparison of two combined vaccines against typhoid fever and hepatitis A in healthy adults. *Vaccine. 2004;23:29–35.*

87. **Rao S, Mao JS, Motlekar S, Fangcheng Z, Kadhe G.** A review of immunogenicity and tolerability of live attenuated Hepatitis A vaccine in children. *Hum Vaccin Immunother. 2016;12:3160–5.*

88. **Ott JJ, Irving G, Wiersma ST.** Long-term protective effects of hepatitis A vaccines. A systematic review. *Vaccine. 2012;31:3–11.*

89. **Funkhouser AW, Purcell RH, D'Hondt E, Emerson SU.** Attenuated hepatitis A virus: genetic determinants of adaptation to growth in MRC-5 cells. *J Virol. 1994;68:148–57.*

90. **Xu ZY, Wang XY.** Live attenuated hepatitis A vaccines developed in China. *Hum Vaccin Immunother. 2014;10:659–66.*

91. **Cohen JI, Rosenblum B, Ticehurst JR, Daemer RJ, Feinstone SM, Purcell RH.** Complete nucleotide sequence of an attenuated hepatitis A virus: comparison with wild-type virus. *Proc Natl Acad Sci U S A. 1987;84:2497–501.*

92. **Jiang CL, Wang PF, Liu JY, Zhang HY, Wan ZJ.** [Genomic sequence of hepatitis A virus L-A-1 vaccine strain]. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2004;18:360–2.*

93. **Chen Y, Yang N, Hong Y.** [Construction of gene library of attenuated liver hepatitis A vaccine (H2 strain)]. *Zhonghua Yi Xue Za Zhi. 1996;76:342–4.*

94. **Lemon SM.** Immunologic approaches to assessing the response to inactivated hepatitis A vaccine. *J Hepatol. 1993;18(Suppl 2):S15–9.*

95. **Lemon SM.** Type A viral hepatitis: epidemiology, diagnosis, and prevention. *Clin Chem. 1997;43:1494–9.*

96. **Morgeaux S, Manniam I, Variot P, Buchheit KH, Daas A, Wierer M et al.** Establishment of hepatitis A vaccine (inactivated, non-adsorbed) BRP batches 2 and 3. *Pharmeur Bio Sci Notes. 2015;2015:118–30.*

97. **Wiedermann G, Ambrosch F, Andre FE, Delem A, D'Hondt E, Safary A.** Thermostability of an inactivated hepatitis A vaccine stored at 37 degrees C for one week. *J Med Virol. 1994;44:442.*

98. **Shin EC, Sung PS, Park SH.** Immune responses and immunopathology in acute and chronic viral hepatitis. *Nat Rev Immunol. 2016;16:509–23.*

99. **Shin SY, Jeong SH, Sung PS, Lee J, Kim HJ, Lee HW et al.** Comparative analysis of liver injury-associated cytokines in acute hepatitis A and B. *Yonsei Med J. 2016;57:652–7.*

100. **Lanford RE, Feng Z, Chavez D, Guerra B, Brasky KM, Zhou Y et al.** Acute hepatitis A virus infection is associated with a limited type I interferon response and persistence of intrahepatic viral RNA. *Proc Natl Acad Sci U S A. 2011;108:11223–8.*

101. **Hirai-Yuki A, Hensley L, McGivern DR, González-López O, Das A, Feng H et al.** MAVS-dependent host species range and pathogenicity of human hepatitis A virus. *Science. 2016;353:1541–5.*

102. **Lemon SM, Brown CD, Brooks DS, Simms TE, Bancroft WH.** Specific immunoglobulin M response to hepatitis A virus determined by solid-phase radioimmunoassay. *Infect Immun. 1980;28:927–36.*

103. **Margolis HS, Nainan OV, Krawczynski K, Bradley DW, Ebert JW, Spelbring J et al.** Appearance of immune complexes during experimental hepatitis A infection in chimpanzees. *J Med Virol. 1988;26:315–26.*

104. **Bower WA, Nainan OV, Han X, Margolis HS.** Duration of viremia in hepatitis A virus infection. *J Infect Dis. 2000;182:12–7.*

105. **Liaw YF, Yang CY, Chu CM, Huang MJ.** Appearance and persistence of hepatitis A IgM antibody in acute clinical hepatitis A observed in an outbreak. *Infection. 1986;14:156–8.*

106. **Kao HW, Ashcavai M, Redeker AG.** The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. *Hepatology. 1984;4:933–6.*

107. **Villarejos VM, Gutierrez-Diermissen A, Anderson-Visona K, Rodriguez-Aragones A, Provost PJ, Hilleman MR.** Development of immunity against hepatitis A virus by subclinical infection. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine. 1976;153:205–8.*

108. **Liu JP, Nikolova D, Fei Y.** Immunoglobulins for preventing hepatitis A. *Cochrane Database Syst Rev 2009:CD004181.*

109. **Rendi-Wagner P, Korinek M, Winkler B, Kundi M, Kollaritsch H, Wiedermann U.** Persistence of seroprotection 10 years after primary hepatitis A vaccination in an unselected study population. *Vaccine. 2007;25:927–31.*

110. **Kabrane-Lazizi Y, Emerson SU, Herzog C, Purcell RH.** Detection of antibodies to HAV 3C proteinase in experimentally infected chimpanzees and in naturally infected children. *Vaccine. 2001;19:2878–83.*

111. **Robertson BH, Jia XY, Tian H, Margolis HS, Summers DF, Ehrenfeld E.** Serological approaches to distinguish immune response to hepatitis A vaccine and natural infection. *Vaccine. 1992;10(Suppl 1):S106–9.*

112. **Stewart DR, Morris TS, Purcell RH, Emerson SU.** Detection of antibodies to the nonstructural 3C proteinase of hepatitis A virus. *J Infect Dis. 1997;176:593–601.*

113. **Sikuler E, Keynan A, Hanuka N, Friedman MG, Sarov I.** Detection and persistence of specific IgA antibodies in serum of patients with hepatitis A by capture radioimmunoassay. *J Med Virol. 1983;11:287–94.*

114. **Locarnini SA, Coulepis AG, Kaldor J, Gust ID.** Coproantibodies in hepatitis A: detection by enzyme-linked immunosorbent assay and immune electron microscopy. *J Clin Microbiol. 1980;11:710–6.*

115. **Stapleton JT, Lange DK, LeDuc JW, Binn LN, Jansen RW, Lemon SM.** The role of secretory immunity in hepatitis A virus infection. *J Infect Dis. 1991;163:7–11.*

116. **Vallbracht A, Maier K, Stierhof YD, Wiedmann KH, Flehmig B, Fleischer B.** Liver-derived cytotoxic T cells in hepatitis A virus infection. *J Infect Dis. 1989;160:209–17.*

117. **Hashimoti E, Kojimahara N, Noguchi S, Taniai M, Ishiguro N, Hayashi N.** Immunohistochemical characterization of hepatic lymphocytes in acute hepatitis A, B, and C. *J Clin Gastroenterol. 1996;23:199–202.*

118. **Sanchez G, Pinto RM, Bosch A.** A novel CD4+ T-helper lymphocyte epitope in the VP3 protein of hepatitis A virus. *J Med Virol. 2004;72:525–32.*

119. **Baba M, Fukai K, Hasegawa H, Nakayabu M, Suzuki S.** The role of natural killer cells and lymphokine activated killer cells in the pathogenesis of hepatic injury in hepatitis A [corrected]. *J Clin Lab Immunol. 1992;38:1–14.*

120. **Vallbracht A, Gabriel P, Maier K, Hartmann F, Steinhardt HJ, Müller C et al.** Cell-mediated cytotoxicity in hepatitis A virus infection. *Hepatology. 1986;6:1308–14.*

121. **Kurane I, Binn LN, Bancroft WH, Ennis FA.** Human lymphocyte responses to hepatitis A virus-infected cells: interferon production and lysis of infected cells. *J Immunol. 1985;135:2140–4.*

122. **Perrella A, Vitiello L, Atripaldi L, Sbreglia C, Grattacaso S, Bellopede P et al.** Impaired function of CD4+/CD25+ T regulatory lymphocytes characterizes the self-limited hepatitis A virus infection. *J Gastroenterol Hepatol. 2008;23:e105–10.*

123. **Schulte I, Hitziger T, Giugliano S, Timm J, Gold H, Heinemann FM et al.** Characterization of CD8+ T-cell response in acute and resolved hepatitis A virus infection. *J Hepatol. 2011;54(2):201–8*

124. **Wunchmann S,Vallbracht A, Flehmig B, Winokur P, Klinzmann D, Stapleton JT.** Cytolytic T-lymphocyte epitopes are present on hepatitis A virus structural proteins.In Viral Hepatitis and Liver Disease. Edts M.Rizzetto, RH Purcell, JL Gerin and G.Verme. *Turin: Edizion: Minerva Medica; 1997 pp51-54*

125. **Yang Y, Liang Y, Qu L, Chen Z, Yi M, Li K et al.** Disruption of innate immunity due to mitochondrial targeting of a picornaviral protease precursor. *Proc Natl Acad Sci U S A. 2007;104:7253–8.*

126. **Qu L, Feng Z, Yamane D, Liang Y, Lanford RE, Li K et al.** Disruption of TLR3 signaling due to cleavage of TRIF by the hepatitis A virus protease-polymerase processing intermediate, 3CD. *PLoS Pathog. 2011;7:e1002169.*

127. **Andre FE.** Universal mass vaccination against hepatitis A. *Curr Top Microbiol Immunol. 2006;304:95–114.*

128. **Van Damme P, Banatvala J, Fay O, Iwarson S, McMahon B, Van Herck K et al.** Hepatitis A booster vaccination: is there a need? *Lancet. 2003;362:1065–71.*

129. **Bovier PA, Bock J, Ebengo TF, Frösner G, Glaus J, Herzog C et al.** Predicted 30-year protection after vaccination with an aluminum-free virosomal hepatitis A vaccine. *J Med Virol. 2010;82:1629–34.*

130. **Shouval D, Ashur Y, Adler R, Lewis JA, Armstrong ME, Davide JP et al.** Single and booster dose responses to an inactivated hepatitis A virus vaccine: comparison with immune serum globulin prophylaxis. *Vaccine. 1993;11 (Suppl 1):S9–14.*

131. **Schmidtke P, Habermehl P, Knuf M, Meyer CU, Sanger R, Zepp F.** Cell mediated and antibody immune response to inactivated hepatitis A vaccine. *Vaccine. 2005;23:5127–32.*

132. **Cederna JB, Klinzman D, Stapleton JT.** Hepatitis A virus-specific humoral and cellular immune responses following immunization with a formalin-inactivated hepatitis A vaccine. *Vaccine. 1999;18:892–8.*

133. **Leroux-Roels G, Desombere I, Van Herck K, Thoelen S, Collard F, Van Damme P.** Long-term persistence of cellular immunity towards hepatitis A virus following hepatitis A virus vaccination. In viral Hepatitis and Liver disease Edts HS Margolis, MJ Alter, TJ Liang and JL Dienstag. *International Medical Press; 2002 pp41-44.*

134. **Garner-Spitzer E, Kundi M, Rendi-Wagner P, Winkler B, Wiedermann G, Holzmann H et al.** Correlation between humoral and cellular immune responses and the expression of the hepatitis A receptor HAVcr-1 on T cells after hepatitis A re-vaccination in high and low-responder vaccinees. *Vaccine. 2009;27:197–204.*

135. **Hayney MS, Buck JM, Muller D.** Production of interferon-gamma and interleukin-10 after inactivated hepatitis A immunization. *Pharmacotherapy. 2003;23:431–5.*

136. **Purcell RH, D'Hondt E, Bradbury R, Emerson SU, Govindarajan S, Binn L.** Inactivated hepatitis A vaccine: active and passive immunoprophylaxis in chimpanzees. *Vaccine. 1992;10(Suppl 1):S148–51.*

137. **Lemon SM, Binn LN.** Serum neutralizing antibody response to hepatitis A virus. *J Infect Dis. 1983;148:1033–9.*

138. **Nalin DR, Kuter BJ, Brown L, Patterson C, Calandra GB, Werzberger A et al.** Worldwide experience with the CR326F-derived inactivated hepatitis A virus vaccine in pediatric and adult populations: an overview. *J Hepatol. 1993;18(Suppl 2):S51–5.*

139. **Clemens R, Safary A, Hepburn A, Roche C, Stanbury WJ, Andre FE.** Clinical experience with an inactivated hepatitis A vaccine. *J Infect Dis. 1995;171(Suppl 1):S44–9.*

140. **Zhang X, An J, Tu A, Liang X, Cui F, Zheng H.** Comparison of immune persistence among inactivated and live attenuated hepatitis a vaccines 2 years after a single dose. *Hum Vaccin Immunother. 2016;12:2322–6.*

141. **Chen Y, Zhou CL, Zhang XJ, Zhang YH, Hao ZY, Wang SM et al.** Immune memory at 17-years of follow-up of a single dose of live attenuated hepatitis A vaccine. *Vaccine. 2018;36:114–21.*

142. **Lopez EL, Contrini MM, Mistchenko A, Debbag R.** Long-term immunity after two doses of inactivated hepatitis A vaccine, in Argentinean children. *Pediatr Infect Dis J. 2010;29:568–70.*

143. **Van Herck K, Van Damme P, Lievens M, Stoffel M.** Hepatitis A vaccine: indirect evidence of immune memory 12 years after the primary course. *J Med Virol. 2004;72:194–6.*

144. **Van Damme P, Leroux-Roels G, Suryakiran P, Folschweiller N, Van Der Meeren O.** Persistence of antibodies 20 y after vaccination with a combined hepatitis A and B vaccine. *Hum Vaccin Immunother. 2017:1–9.*

145. **Melgaco JG, Morgado LN, Santiago MA, Mendes de Oliveira J, Lewis-Ximenez LL, Hasselmannd B et al.** A single dose of inactivated hepatitis A vaccine promotes HAV-specific memory cellular response similar to that induced by a natural infection. *Vaccine. 2015;33:3813–20.*

146. **Vacchino MN.** Incidence of hepatitis A in Argentina after vaccination. *J Viral Hepat. 2008;15(Suppl 2):47–50.*

147. **Nolan T, Bernstein H, Blatter MM, Bromberg K, Guerra F, Kennedy W et al.** Immunogenicity and safety of an inactivated hepatitis A vaccine administered concomitantly with diphtheria-tetanus-acellular pertussis and haemophilus influenzae type B vaccines to children less than 2 years of age. *Pediatrics. 2006;118:e602–9.*

148. **Stokes J Jr, Blanchard M, Neefe JR et al.** Methods of protection against homologous serum hepatitis; studies on the protective value of gamma globulin in homologous serum hepatitis Sh virus. *JAMA. 1948;138:336–41.*

149. **Stapleton JT.** Passive immunization against hepatitis A. *Vaccine. 1992; 10 (Suppl 1):S45–7.*

150. **Gluud LL, Gluud C.** Meta-analyses on viral hepatitis. *Infect Dis Clin North Am. 2009;23:315–30.*

151. **Cohn EJ, Oncley JL, Strong LE, Hughes WL, Armstrong SH.** Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J Clin Invest. 1944;23:417–32.*

152. **Higgins G, Wreghitt TG, Gray JJ, Blagdon J, Taylor CE.** Hepatitis A virus antibody in East Anglian blood donors. *Lancet. 1990;336:1330.*

153. **Ferguson M, Sands D, Lelie N.** Hepatitis A immunoglobulin: an international collaborative study to establish the second international standard. *Biologicals. 2000;28:233–40.*

154. **Tejada-Strop A, Costafreda MI, Dimitrova Z, Kaplan GG, Teo CG.** Evaluation of potencies of immune globulin products against hepatitis A. *JAMA Intern Med. 2017;177:430–2.*

155. **Hall WT, Madden DL, Mundon FK, Brandt DE, Clarke NA.** Protective effect of immune serum globulin (ISG) against hepatitis A infection in a natural epidemic. *Am J Epidemiol. 1977;106:72–5.*

156. **Victor JC, Monto AS, Surdina TY, Suleimenova SZ, Vaughan G, Nainan OV et al.** Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med. 2007;357:1685–94.*

157. **Lemon SM, Murphy PC, Provost PJ, Chalikonda I, Davide JP, Schofield TL et al.** Immunoprecipitation and virus neutralization assays demonstrate qualitative differences between protective antibody responses to inactivated hepatitis A vaccine and passive immunization with immune globulin. *J Infect Dis. 1997;176:9–19.*

158. **Wagner G, Lavanchy D, Darioli R, Pécoud A, Brulein V, Safary A et al.** Simultaneous active and passive immunization against hepatitis A studied in a population of travellers. V*accine. 1993;11:1027–32.*

159. **Walter EB, Hornick RB, Poland GA, Tucker R, Bland CL, Clements DA et al.** Concurrent administration of inactivated hepatitis A vaccine with immune globulin in healthy adults. *Vaccine. 1999;17:1468–73.*

160. **Howell D, Barbara JA, Brennan M.** Hepatitis A virus, blood donors, and immunoglobulin preparations. *Lancet. 1991;337:1165.*

161. **Tedder RS, Cameron CH, Barbara JA, Howell D.** Viral hepatitis markers in blood donors with history of jaundice. *Lancet. 1980;1:595–6.*

162. **Anonychuk AM, Tricco AC, Bauch CT, Pham B, Gilca V, Duval B et al.** Cost-effectiveness analyses of hepatitis A vaccine: a systematic review to explore the effect of methodological quality on the economic attractiveness of vaccination strategies. *Pharmacoeconomics. 2008;26:17–32.*

163. **Soysal A, Gokce I, Pehlivan T, Bakir M.** Interchangeability of a hepatitis A vaccine second dose: Avaxim 80 following a first dose of Vaqta 25 or Havrix 720 in children in Turkey. *Eur J Pediatr. 2007;166:533–9.*

164. **Abarca K, Ibanez I, Perret C, Vial P, Zinsou JA.** Immunogenicity, safety, and interchangeability of two inactivated hepatitis A vaccines in Chilean children. *Int J Infect Dis. 2008;12:270–7.*

165. **Dagan R, Amir J, Livni G, Greenberg D, Abu-Abed J, Guy L et al.** Concomitant administration of a virosome-adjuvanted hepatitis a vaccine with routine childhood vaccines at age twelve to fifteen months: a randomized controlled trial. *Pediatr Infect Dis J. 2007;26:787–93.*

166. **Prikazsky V, Olear V, Cernoch A, Safary A, Andre FE.** Interruption of an outbreak of hepatitis A in two villages by vaccination. *J Med Virol. 1994;44:457–9.*

167. **Sagliocca L, Amoroso P, Stroffolini T, Adamo B, Tosti ME, Lettieri G et al.** Efficacy of hepatitis A vaccine in prevention of secondary hepatitis A infection: a randomised trial. *Lancet. 1999;353:1136–9.*

168. **Zamir C, Rishpon S, Zamir D, Leventhal A, Rimon N, Ben-Porath E.** Control of a community-wide outbreak of hepatitis A by mass vaccination with inactivated hepatitis A vaccine. *Eur J Clin Microbiol Infect Dis. 2001;20:185–7.*

169. **Selnikova O, Moisseeva A, Zadorozhnaja V, Dokachenko A, Kachur N, Gavrik S.** Hepatitis A vaccination effectiveness during an outbreak in the Ukraine. *Vaccine. 2008;26:3135–7.*

170. Update: Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR. 2007;56:1080–4.*

171. **Craig AS, Sockwell DC, Schaffner W, Moore WL Jr, Skinner JT, Williams IT et al.** Use of hepatitis A vaccine in a community-wide outbreak of hepatitis A. *Clin Infect Dis. 1998;27:531–5.*

172. **Franceschi C, Monti D, Barbieri D, Grassilli E, Troiano L, Salvioli S et al.** Immunosenescence in humans: deterioration or remodelling? *Int Rev Immunol. 1995;12:57–74.*

173. **Ben-Yehuda A, Weksler ME.** Immune senescence: mechanisms and clinical implications. *Cancer Invest. 1992;10:525–31.*

174. **Genton B, D'Acremont V, Furrer HJ, Hatz C, Louis L.** Hepatitis A vaccines and the elderly. *Travel Med Infect Dis. 2006;4:303–12.*

175. **Scheifele DW, Bjornson GJ.** Evaluation of inactivated hepatitis A vaccine in Canadians 40 years of age or more. *CMAJ. 1993;148:551–5.*

176. **Reuman PD, Kubilis P, Hurni W, Brown L, Nalin D.** The effect of age and weight on the response to formalin inactivated, alum-adjuvanted hepatitis A vaccine in healthy adults. *Vaccine. 1997;15:1157–61.*

177. **Steffen R, Kane MA, Shapiro CN, Billo N, Schoellhorn KJ, van Damme P.** Epidemiology and prevention of hepatitis A in travelers. *JAMA. 1994;272:885–9.*

178. **Holzer BR, Hatz C, Schmidt-Sissolak D, Gluck R, Althaus B, Egger M.** Immunogenicity and adverse effects of inactivated virosome versus alum-adsorbed hepatitis A vaccine: a randomized controlled trial. *Vaccine. 1996;14:982–6.*

179. **D'Acremont V, Herzog C, Genton B.** Immunogenicity and safety of a virosomal hepatitis A vaccine (Epaxal) in the elderly. *J Travel Med. 2006;13:78–83.*

180. **Van Der Meeren O, Crasta P, de Ridder M.** A retrospective pooled analysis assessing the effect of age on the immunogenicity of Havrix in healthy adults. *Hum Vaccin Immunother. 2015;11:1729–34.*

181. **Maiwald H, Jilg W, Bock HL, Loscher T, Sonnenburg F.** Long-term persistence of anti-HAV antibodies following active immunization with hepatitis A vaccine. *Vaccine. 1997;15:346–8.*

182. **Launay O, Grabar S, Gordien E, Desaint C, Jegou D, Abad S et al.** Immunological efficacy of a three-dose schedule of hepatitis A vaccine in HIV-infected adults: HEPAVAC study. *J Acquir Immune Defic Syndr. 2008;49:272–5.*

183. **Lieberman JM, Chang SJ, Partridge S, Hollister JC, Kaplan KM, Jensen EH et al.** Kinetics of maternal hepatitis a antibody decay in infants: implications for vaccine use. *Pediatr Infect Dis J. 2002;21:347–8.*

184. **Usonis V, Bakasenas V, Valentelis R, Katiliene G, Vidzeniene D, Herzog C.** Antibody titres after primary and booster vaccination of infants and young children with a virosomal hepatitis A vaccine (Epaxal). *Vaccine. 2003;21:4588–92.*

185. **Letson GW, Shapiro CN, Kuehn D, Gardea C, Welty TK, Krause DS et al.** Effect of maternal antibody on immunogenicity of hepatitis A vaccine in infants. *The J Pediatr. 2004;144:327–32.*

186. **Dagan R, Amir J, Mijalovsky A, Kalmanovitch I, Bar-Yochai A, Thoelen S et al.** Immunization against hepatitis A in the first year of life: priming despite the presence of maternal antibody. *Pediatr Infect Dis J. 2000;19:1045–52.*

187. **Fiore AE, Shapiro CN, Sabin K, Labonte K, Darling K, Culver D et al.** Hepatitis A vaccination of infants: effect of maternal antibody status on antibody persistence and response to a booster dose. *Pediatr Infect Dis J. 2003;22:354–9.*

188. **Fonquernie L, Meynard JL, Charrois A, Delamare C, Meyohas MC, Frottier J.** Occurrence of acute hepatitis A in patients infected with human immunodeficiency virus. *Clin Infect Dis. 2001;32:297–9.*

189. **Ida S, Tachikawa N, Nakajima A, Daikoku M, Yano M, Kikuchi Y et al.** Influence of human immunodeficiency virus type 1 infection on acute hepatitis A virus infection. *Clin Infect Dis. 2002;34:379–85.*

190. **Costa-Mattioli M, Allavena C, Poirier AS, Billaudel S, Raffi F, Ferre V.** Prolonged hepatitis A infection in an HIV-1 seropositive patient. *J Med Virol. 2002;68:7–11.*

191. **Ridolfo AL, Rusconi S, Antinori S, Balotta C, Galli M.** Persisting HIV-1 replication triggered by acute hepatitis A virus infection. *Antivir Ther. 2000;5:15–7.*

192. **Wallace MR, Hill HE, Tasker SA, Miller LK.** Hepatitis A in human immunodeficiency virus-infected patients. *Clin Infect Dis. 1998;27:651–3.*

193. **Laurence JC.** Hepatitis A and B immunizations of individuals infected with human immunodeficiency virus. A*m J Med. 2005;118(Suppl 10ª):75S–83S.*

194. **Overton ET, Nurutdinova D, Sungkanuparph S, Seyfried W, Groger RK, Powderly WG.** Predictors of immunity after hepatitis A vaccination in HIV-infected persons. *J Viral Hepat. 2007;14:189–93*.

195. **Mena G, Garcia-Basteiro AL, Bayas JM.** Hepatitis B and A vaccination in HIV-infected adults: A review. *Hum Vaccin Immunother. 2015;11:2582–98.*

196. **Gunther M, Stark K, Neuhaus R, Reinke P, Schroder K, Bienzle U.** Rapid decline of antibodies after hepatitis A immunization in liver and renal transplant recipients. *Transplantation. 2001;71:477–9.*

197. **Stark K, Gunther M, Neuhaus R, Reinke P, Schröder K, Linnig S et al.** Immunogenicity and safety of hepatitis A vaccine in liver and renal transplant recipients. *J Infect Dis. 1999;180:2014–7.*

198. **Arslan M, Wiesner RH, Poterucha JJ, Zein NN.** Safety and efficacy of hepatitis A vaccination in liver transplantation recipients. *Transplantation. 2001;72:272–6.*

199. **Godoi ER, de Souza VA, Cakmak S, Machado AF, Vilas Boas LS, Machado CM.** Loss of hepatitis A virus antibodies after bone marrow transplantation. *Bone Marrow Transplant. 2006;38:37–40.*

200. **Vento S, Garofano T, Renzini C, Cainelli F, Casali F, Ghironzi G et al.** Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *New Engl J Med. 1998;338:286–90.*

201. **Pramoolsinsap C, Poovorawan Y, Hirsch P, Busagorn N, Attamasirikul K.** Acute, hepatitis-A super-infection in HBV carriers, or chronic liver disease related to HBV or HCV. *Ann Trop Med Parasitol. 1999;93:745–51.*

202. **Smallwood GA, Coloura CT, Martinez E, Stieber AC, Heffron TG.** Can patients awaiting liver transplantation elicit an immune response to the hepatitis A vaccine? *Transplant Proc. 2002;34:3289–90.*

203. **Lee SD, Chan CY, Yu MI, Wang YJ, Chang FY, Lo KJ et al.** Safety and immunogenicity of inactivated hepatitis A vaccine in patients with chronic liver disease. *J Med Virol. 1997;52:215–8.*

204. **Majda-Stanislawska E, Bednarek M, Kuydowicz J.** Immunogenicity of inactivated hepatitis A vaccine in children with chronic liver disease. *Pediatr Infect Dis J. 2004;23:571–4.*

205. **Ferreira CT, da Silveira TR, Vieira SM, Taniguchi A, Pereira-Lima J.** Immunogenicity and safety of hepatitis A vaccine in children with chronic liver disease. *J Pediatr Gastroenterol Nutr. 2003;37:258–61.*

206. **Dumot JA, Barnes DS, Younossi Z, et al.** Immunogenicity of hepatitis A vaccine in decompensated liver disease. *Am J Gastroenterol. 1999;94:1601–4.*

207. **Gil A, Gonzalez A, Dal-Re R, Calero JR.** Interference assessment of yellow fever vaccine with the immune response to a single-dose inactivated hepatitis A vaccine (1440 EL.U.). A controlled study in adults. *Vaccine. 1996;14:1028–30.*

208. **Guerra FA, Gress J, Werzberger A, Reisinger K, Walter E, Lakkis H et al.** Safety, tolerability and immunogenicity of VAQTA given concomitantly versus nonconcomitantly with other pediatric vaccines in healthy 12-month-old children. *Pediatr Infect Dis J. 2006;25:912–9.*

209. **Bienzle U, Bock HL, Kruppenbacher JP, Hofmann F, Vogel GE, Clemens R.** Immunogenicity of an inactivated hepatitis A vaccine administered according to two different schedules and the interference of other "travellers" vaccines with the immune response. *Vaccine. 1996;14:501–5.*

210. **Jong EC, Kaplan KM, Eves KA, Taddeo CA, Lakkis HD, Kuter BJ.** An open randomized study of inactivated hepatitis A vaccine administered concomitantly with typhoid fever and yellow fever vaccines. *J Travel Med. 2002;9:66–70.*

211. **Usonis V, Meriste S, Bakasenas V, Lutsar I, Collard F, Stoffel M et al.** Immunogenicity and safety of a combined hepatitis A and B vaccine administered concomitantly with either a measles-mumps-rubella or a diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis vaccine mixed with a Haemophilus influenzae type b conjugate vaccine in infants aged 12-18 months. *Vaccine. 2005;23:2602–6.*

212. **Ambrosch F, Fritzell B, Gregor J, Jonas S, Kollaritsch H, Teulieres L et al.** Combined vaccination against yellow fever and typhoid fever: a comparative trial. *Vaccine. 1994;12:625–8.*

213. **Overbosch D, Peyron F, Picot N, Varichon JP, Dumas R, Chambonneau L et al.** Combined typhoid fever and hepatitis A vaccine: comparison of immunogenicity and safety to concomitant monovalent vaccine over 3 years. *J Travel Med. 2005;12:319–26.*

214. **Beran J, Chlibek R, Weber F. A** combined dual-chamber typhoid/hepatitis A vaccine as a booster dose in hepatitis A primed adults. *Vaccine. 2003;21:4650–4.*

215. **Proell S, Maiwald H, Nothdurft HD, Saenger R, Vollmar J, De Clercq N et al.** Combined vaccination against hepatitis A, hepatitis B, and typhoid fever: safety, reactogenicity, and immunogenicity. *J Travel Med. 2002;9:122–6.*

216. **Loebermann M, Kollaritsch H, Ziegler T, Rendi-Wagner P, Chambonneau L, Dumas R et al.** A randomized, open-label study of the immunogenicity and reactogenicity of three lots of a combined typhoid fever/hepatitis A vaccine in healthy adults. *Clin Ther. 2004;26:1084–91.*

217. **Bryan NP, Hastie KJ, Chapple CR.** Randomised prospective trial of contact laser prostatectomy (CLAP) versus visual laser coagulation of the prostate (VLAP) for the treatment of benign prostatic hyperplasia. 2-year follow-up. *Eur Urol. 2000;38:265–71.*

218. **Bovier PA, Farinelli T, Loutan L.** Interchangeability and tolerability of a virosomal and an aluminum-adsorbed hepatitis A vaccine. *Vaccine. 2005;23:2424–9.*

219. **Ekwall E, Ulgemo A, Watson M, Boisnard F, Thomas S, Goullet F.** Interchangeability of hepatitis A boosters, Avaxim and Vaqta, in healthy adults following a primary dose of the combined typhoid/Hepatitis A vaccine Viatim. *Vaccine. 2006;24:4450–7.*

220. **Werzberger A, Mensch B, Nalin DR, Kuter BJ.** Effectiveness of hepatitis A vaccine in a former frequently affected community: 9 years' followup after the Monroe field trial of VAQTA. *Vaccine. 2002;20:1699–701.*

221. **Bovier PA.** Epaxal: a virosomal vaccine to prevent hepatitis A infection. *Expert Rev Vaccines. 2008;7:1141–50.*

222. **Dagan R, Leventhal A, Anis E, Slater P, Ashur Y, Shouval D.** Incidence of hepatitis A in Israel following universal immunization of toddlers. *JAMA. 2005;294:202–10.*

223. **Bialek SR, Thoroughman DA, Hu D, Simard EP, Chattin J, Cheek J et al.** Hepatitis A incidence and hepatitis a vaccination among American Indians and Alaska Natives, 1990-2001. *Am J Public Health. 2004;94:996–1001.*

224. **Wasley A, Samandari T, Bell BP.** Incidence of hepatitis A in the United States in the era of vaccination. *JAMA 2005;294:194–201.*

225. **Kushel M.** Hepatitis A Outbreak in California - addressing the root cause. *N Engl J Med. 2018;378:211–3.*

226. **Beebeejaun K, Degala S, Balogun K, Simms I, Woodhall SC, Heinsbroek E et al.** Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017. *Euro Surveill. 2017;22.*

227. **Freidl GS, Sonder GJ, Bovee LP, Friesema IH, van Rijckevorsel GG, Ruijs WL et al.** Hepatitis A outbreak among men who have sex with men (MSM) predominantly linked with the EuroPride, the Netherlands, July 2016 to February 2017. *Euro Surveill. 2017;22.*

228. **Cheng CY, Wu HH, Zou H, Lo YC.** Epidemiological characteristics and associated factors of acute hepatitis A outbreak among HIV-coinfected men who have sex with men in Taiwan, June 2015–December 2016. *J Viral Hepat. 2018;25:1208–15.*

229. **Werber D, Michaelis K, Hausner M, Sissolak D, Wenzel J, Bitzegeio J et al.** Ongoing outbreaks of hepatitis A among men who have sex with men (MSM), Berlin, November 2016 to January 2017 - linked to other German cities and European countries. *Euro Surveill. 2017;22.*

230. **Gozlan Y, Bar-Or I, Rakovsky A, Savion M, Amitai Z, Sheffer R et al.** Ongoing hepatitis A among men who have sex with men (MSM) linked to outbreaks in Europe in Tel Aviv area, Israel, December 2016–June 2017. *Euro Surveill. 2017;22.*

231. **Lanini S, Minosse C, Vairo F, et al.** A large ongoing outbreak of hepatitis A predominantly affecting young males in Lazio, Italy; August 2016–March 2017. *PLoS One. 2017;12:e0185428.*

232. **Lopalco PL, Salleras L, Barbuti S, Germinario C, Bruguera M, Buti M et al.** Hepatitis A and B in children and adolescents – what can we learn from Puglia (Italy) and Catalonia (Spain)? *Vaccine 2000;19:470–4.*

233. **Dominguez A, Salleras L, Carmona G, Batalla J.** Effectiveness of a mass hepatitis A vaccination program in preadolescents. *Vaccine. 2003;21:698–701.*

234. **Hanna JN, Hills SL, Humphreys JL.** Impact of hepatitis A vaccination of Indigenous children on notifications of hepatitis A in north Queensland. *Med J Aust. 2004;181:482–5.*

235. **Manor Y, Lewis M, Ram D, Daudi N, Mor O, Savion M et al.** Evidence for Hepatitis A virus endemic circulation in Israel despite universal toddlers' vaccination since 1999 and low clinical incidence in all age groups. *J Infect Dis. 2016.*

236. **Levine H, Kopel E, Anis E, Givon-Lavi N, Dagan R.** The impact of a national routine immunisation programme initiated in 1999 on Hepatitis A incidence in Israel, 1993 to 2012. *Euro Surveill. 2015;20:3–10.*

237. **Stuurman AL, Marano C, Bunge EM, De Moerlooze L, Shouval D.** Impact of universal mass vaccination with monovalent inactivated hepatitis A vaccines – a systematic review. *Hum Vaccin Immunother. 2017;13:724–36.*

238. **Cervio G, Trentadue J, D'Agostino D, Luque C, Giorgi M, Armon J et al.** Decline in HAV-associated fulminant hepatic failure and liver transplant in children in Argentina after the introduction of a universal hepatitis A vaccination program. *Hepat Med. 2011;3:99–106.*

239. **Van Damme P, Mathei C, Thoelen S, Meheus A, Safary A, Andre FE.** Single dose inactivated hepatitis A vaccine: rationale and clinical assessment of the safety and immunogenicity. *J Med Virol. 1994;44:435–41.*

240. **Beck BR, Hatz C, Bronnimann R, Herzog C.** Successful booster antibody response up to 54 months after single primary vaccination with virosome-formulated, aluminum-free hepatitis A vaccine. *Clin Infect Dis. 2003;37:e126–8.*

241. **Iwarson S, Lindh M, Widerstrom L.** Excellent booster response 4–6 y after a single primary dose of an inactivated hepatitis A vaccine. *Scand J Infect Dis. 2002;34:110–1.*

242. **Espul C, Benedetti L, Linares M, Cuello H, Lo Castro I, Thollot Y et al.** Seven-year follow-up of the immune response after one or 2 doses of inactivated hepatitis A vaccine given at 1 year of age in the Mendoza Province of Argentina. *Hum Vaccin Immunother. 2017:1–6.*

243. **Mayorga O, Buhler S, Jaeger VK, Bally S, Hatz C, Frösner G et al.** Single-dose hepatitis A immunization: 7.5-year observational pilot study in Nicaraguan children to assess protective effectiveness and humoral immune memory response. *J Infect Dis. 2016;214:1498–506.*

244. **Song YJ, Lim J, Park WS, Sohn H, Lee MS, Shin DH et al.** Seropositivity among Korean young adults approximately 2 years after a single-dose vaccination against hepatitis A virus. *PLoS One. 2015;10:e0142297.*

245. **Zhang Z, Zhu X, Hu Y, Liang M, Sun J, Song Y et al.** Five-year antibody persistence in children after one dose of inactivated or live attenuated hepatitis A vaccine. *Hum Vaccin Immunother. 2017;13:1–6.*

246. **Bhave S, Sapru A, Bavdekar A, Kapatkar V, Mane A.** Long-term immunogenicity of single dose of live attenuated hepatitis A vaccine in Indian children. *Indian Pediatr. 2015;52:687–90.*

247. **Mitra M, Shah N, Faridi M, Ghosh A, Sankaranarayanan VS, Aggarwal A et al.** Long term follow-up study to evaluate immunogenicity and safety of a single dose of live attenuated hepatitis a vaccine in children. *Hum Vaccin Immunother. 2015;11:1147–52.*

248. **Ellis A, Ruttimann RW, Jacobs RJ, Meyerhoff AS, Innis BL.** Cost-effectiveness of childhood hepatitis A vaccination in Argentina: a second dose is warranted. *Revista panamericana de salud publica/Pan American Journal of Public Health 2007;21:345–56.*

249. **Farfour E, Lesprit P, Chan Hew Wai A, Mazaux L, Fourn E, Majerholc C et al.** Acute hepatitis A breakthrough in MSM in Paris area: implementation of targeted hepatitis A virus vaccine in a context of vaccine shortage. *AIDS. 2018;32:531–2.*

250. **Rodriguez-Tajes S, Perpinan E, Caballol B, Lens S, Mariño Z, Costa J et al.** Hepatitis A outbreak in Barcelona among men who have sex with men (MSM), January-June 2017: A hospital perspective. *Liver Int. 2018;38:588–93.*

251. **Petersen J, Freedman J, Ford L, Gawthrop M, Simons H, Edelstein M et al.** Changes to country-specific hepatitis A travel vaccination recommendation for UK travellers in 2017 – responding to a vaccine shortage in the national context. *Public Health. 2019;168:150–6.*

252. **Van Der Wielen M, Vertruyen A, Froesner G, Ibáñez R, Hunt M, Herzog C et al.** Immunogenicity and safety of a pediatric dose of a virosome-adjuvanted hepatitis A vaccine: a controlled trial in children aged 1-16 years. *Pediatr Infect Dis J. 2007;26:705–10.*

253. **Niu MT, Salive M, Krueger C, Ellenberg SS.** Two-year review of hepatitis A vaccine safety: data from the Vaccine Adverse Event Reporting System (VAERS). *Clin Infect Dis. 1998;26:1475–6.*

254. **Riedemann S, Reinhardt G, Frosner GG, Ibarra H, Moraleda L, Hering V et al.** Placebo-controlled efficacy study of hepatitis A vaccine in Valdivia, Chile. *Vaccine. 1992;10(Suppl 1):S152–5.*

255. **Ellerbeck EF, Lewis JA, Nalin D, Gershman K, Miller WJ, Armstrong ME et al.** Safety profile and immunogenicity of an inactivated vaccine derived from an attenuated strain of hepatitis A. *Vaccine. 1992;10:668–72.*

256. **Demicheli V, Tiberti D.** The effectiveness and safety of hepatitis A vaccine: a systematic review. *Vaccine. 2003;21:2242–5.*

257. **Black S, Shinefield H, Hansen J, Lewis E, Su L, Coplan P.** A post-licensure evaluation of the safety of inactivated hepatitis A vaccine (VAQTA, Merck) in children and adults. *Vaccine. 2004;22:766–72.*

258. **Shouval D.** Module 18: Hepatitis A. In: The immunological basis for immunization series. *Geneva: World Health Organization; 2010.*

9 789241 516327

## Department of Immunization, Vaccines and Biologicals

**World Health Organization**
20, Avenue Appia
CH-1211 Geneva 27, Switzerland
vaccines@who.int
http://www.who.int/immunization/en/

World Health Organization