From Department of Medical Epidemiology and Biostatistics
Karolinska Institutet, Stockholm, Sweden

EPIDEMIOLOGY, PREVENTION AND CONTROL OF HEPATITIS A VIRUS INFECTION IN THE EUROPEAN UNION

Ettore Severi

Karolinska Institutet

Stockholm 2022

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2022

ISBN number 978-91-8016-799-4

Epidemiology, prevention and control of hepatitis A virus infection in the European Union

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Ettore Severi

The thesis will be defended in public at Lecture Hall Atrium, Nobels väg 12B, Campus Solna, Karolinska Institutet, on Monday, 7 November, 2022 at 13:00

Principal Supervisor:
Professor Emeritus Johan Giesecke
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Co-supervisor(s):
Docent Lisen Arnheim Dahlström
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Professor Pierluigi Lopalco
Universitá del Salento
Faculty of Medicine

Opponent:
Professor Steen Ethelberg
University of Copenhagen
Department of Global Health

Examination Board:
Professor Soo Aleman
Karolinska Institutet
Department of Infectious Diseases

Professor Emeritus Marc Struelens
Université Libre de Bruxelles
Faculty of Medicine

Docent Amelie Plymoth
Karolinska Institutet
Department of Medical Epidemiology and Biostatistics

Ai miei cari

ABSTRACT

Hepatitis A is an acute liver disease caused by the hepatitis A virus (HAV) and transmitted via the faecal-oral route through person-to-person transmission, contaminated food or water. The frequency and severity of symptoms increases with age, with the elderlies and patients with other liver disease at risk of hospitalisation, acute liver failure and death. Hepatitis A is a notifiable disease in the countries of the European Union (EU) and European Economic Area (EEA), and hepatitis A notifications are reported to The European Surveillance System (TESSy). The World Health Organization (WHO) defines the EU/EEA as an area at very low or low HAV endemicity; however, large differences between EU/EEA countries exist. Hepatitis A vaccines are safe and effective. In most countries hepatitis A vaccination is recommended only for groups at increased risk of infection or at increased risk of severe disease. In recent years, large multicountry hepatitis A outbreaks associated with contaminated food or with men who have sex with men (MSM) engaging in sexual practices facilitating faecal-oral transmission have been reported. This thesis' aim was to describe the EU/EEA epidemiology of hepatitis A and provide recommendations on strategies to prevent, monitor and control this evolving public health threat.

In Study 1, we systematically searched the literature for seroprevalence studies performed in EU/EEA countries from 1975 to 2014 and pooled age-specific seroprevalence estimates to obtain estimates of historical HAV incidence and current endemicity. Based on age-specific seroprevalence estimates in adults from 2000 to 2014, we created four HAV susceptibility profiles, paving the way to meaningful grouping of EU/EEA countries in the analysis of Study 4.

HAV is prone to foodborne outbreaks. In Study 2, we described the largest hepatitis A foodborne outbreak reported in the EU/EEA taking place in 2013 and 2014. HAV genome sequencing was an essential tool to link apparently unrelated cases. The multicountry investigation showed the vulnerability of the EU/EEA single food market and that large cross-border foodborne outbreaks can be associated with a significant proportion of hospitalised cases.

In Study 3 we confirmed that a multistrain HAV infection outbreak was underway in the EU/EEA in 2016 and 2017 and that it was disproportionally affecting male patients engaging in high-risk sexual practices. Through a case-case study comparing cases infected with the different outbreak strains we identified no differences in case's exposures. The investigation highlighted the limited uptake of vaccination in a group that should be a priority target.

In study 4, we used hepatitis A surveillance data from TESSy from 2010 to 2019 to describe the epidemiology of hepatitis A in the different EU/EEA areas, place the large foodborne and person-to-person transmission outbreaks in context, and highlight the limitation of hepatitis A surveillance in Europe.

Because of the increasing HAV susceptibility and the risk of more severe disease in older people, it has been hypothesised that the clinical presentation of hepatitis A is worsening. In study 5, we analysed hepatitis A notifications and hospitalisations from 1995 to 2015. Although detecting an increase in the median age at infection, we did not identify an increase in the proportion of hospitalisations associated with clinically severe disease. In this study we also confirmed that older patients and patients with co-morbidities were at increased risk of clinically severe disease.

Based on our study results, we recommend monitoring HAV endemicity and susceptibility in the general EU/EEA population and in MSM. The efforts to vaccinate groups at increased risk of infection should be pre-emptively scaled up without waiting for large outbreaks. Harmonised HAV genome sequencing should be performed at high rates and in all countries, with consequent sharing of sequencing information to rapidly alert on HAV cross-border circulation and enhance early detection of outbreaks. When such outbreaks are detected, multicountry cross-sectorial investigations are paramount for rapid outbreak control. At all times, surveillance should be strengthened with complete and high-quality collection of information about travel history and transmission route. Last, to monitor negative trends in the hepatitis A clinical presentation, better linkage of death and liver transplant registries and surveillance data should be achieved, particularly in those countries that experienced a recent epidemiological transition.

LIST OF SCIENTIFIC PAPERS

I. Carrillo-Santisteve P, Tavoschi L, **Severi E**, Bonfigli S, Edelstein M, Byström E, Lopalco PL, ECDC HAV Expert Panel. Seroprevalence and susceptibility to hepatitis A in the European Union and European Economic Area: a systematic review. The Lancet Infectious Diseases. 2017;17(10).

II. **Severi E**, Verhoef L, Thornton L, Guzman-Herrador BR, Faber M, Sundqvist L, Rimhanen-Finne R, Roque-Afonso AM, Ngui SL, Allerberger F, Baumann-Popczyk A, Muller L, Parmakova K, Alfonsi V, Tavoschi L, Vennema H, Fitzgerald M, Myrmel M, Gertler M, Ederth J, Kontio M, Vanbockstael C, Mandal S, Sadkowska-Todys M, Tosti ME, Schimmer B, O Gorman J, Stene-Johansen K, Wenzel JJ, Jones G, Balogun K, Ciccaglione AR, O' Connor L, Vold L, Takkinen J, Rizzo C. Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014. Eurosurveillance. 2015;20(29).

III. Ndumbi P, Freidl GS, Williams CJ, Mårdh O, Varela C, Avellón A, Varela, C, Avellón A, Friesema I, Vennema H, Beebeejaun K, Ngui SL, Edelstein M, Smith-Palmer A, Murphy N, Dean J, Faber M, Wenzel J, Kontio M, Müller L, Midgley SE, Sundqvist L, Ederth JL, Roque-Afonso AM, Couturier E, Klamer S, Rebolledo J, Suin V, Aberle SW, Schmid D, De Sousa R, Augusto GF, Alfonsi V, Del Manso M, Ciccaglione AR, Mellou K, Hadjichristodoulou C, Donachie A, Borg ML, Sočan M, Poljak M, **Severi E**, Members of the European Hepatitis A Outbreak Investigation Team. Hepatitis A outbreak disproportionately affecting men who have sex with men (MSM) in the European Union and European Economic Area, June 2016 to May 2017. Eurosurveillance. 2018;23(33).

IV. **Severi E**, Tavoschi L, Carrillo-Santisteve P, Westrell T, Marrone G, Giesecke JI, Lopalco PL. Hepatitis A notifications in the EU/EEA from 2010 to 2019: what can we learn from case reporting to the European Surveillance System? (Submitted).

V. **Severi E**, Georgalis L, Pijnacker R, Veneti L, Turiac IA, Chiesa F, Rizzo C, Martinelli D, Vold L, Herrador BG, Martinez CV, Sanchez EVM, Semenza JC, Lopalco P, Dahlström LA, Giesecke Jl. Severity of the clinical presentation of hepatitis A in five European countries from 1995 to 2014. International Journal of Infectious Diseases. 2022;118:34-43.

CONTENTS

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
CI	Confidence interval
DALY	Disability-adjusted life-year
EBS	Event-based surveillance
ECDC	European Centre for Disease Prevention and Control
EU/EEA	European Union/European Economic Area
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
IBS	Indicator-based surveillance
ICD-9	International Statistical Classification of Diseases and Related Health Problems – 9th revision
ICD-10	International Statistical Classification of Diseases and Related Health Problems – 10th revision
Ig	Human immune serum globulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRR	Incidence rate ratio
LISA	Longitudinal integrated database for health insurance and labour market studies
MSM	Men who have sex with men
OR	Odds ratio
PeP	Post-exposure prophylaxis
PWUD	People who use drugs
RNA	Ribonucleic acid
TESSy	The European Surveillance System
RT-PCR	Reverse transcription polymerase chain reaction
WGS	Whole genome sequencing
WHO	World Health Organization
WHO/Europe	World Health Organization Regional Office for Europe

1 INTRODUCTION

The 2019 Global Burden of Disease Study ranks hepatitis A virus infection as the most common acute viral infection in the world. Sub-Saharan Africa and Asia bear the highest incidence of the infection and large part of the associated mortality (1). In the European Union (EU) / European Economic Area (EEA) hepatitis A has different epidemiological patterns, ranging from very low notification rates (<1 case per 100 000 population) in northern and some western EU/EEA countries to much higher rates (>20 cases per 100 000 population) in some eastern EU countries. However, most EU/EEA countries are susceptible to large hepatitis A outbreaks associated with major increases of cases. Hepatitis A is a notifiable disease in the EU/EEA countries and EU/EEA surveillance is framed and applied by the European Centre for Disease Prevention and Control (ECDC) (2).

Hepatitis A virus infection is caused by the hepatitis A virus (HAV). HAV is a small RNA virus characterised by a well-conserved genome. Infection generally happens via the faecal-oral route, through ingestion of the virus. HAV can survive in the environment for long and is resistant to acidification and freezing. These characteristics makes it prone to outbreaks associated with contaminated food and water or person-to-person transmission, often in connection with high-risk sexual practices (3).

HAV infection is only acute and provides immunity for life. The infection can have multiple outcomes: whilst it is mostly asymptomatic in children, the proportion of symptomatic cases and the severity of the clinical hepatitis A presentation increases with age, with older adults and vulnerable patients at higher risk of acute viral disease and hepatic injury, which can culminate in fulminant hepatitis and, in some instances, death (4). In settings of prolonged low endemicity, like in many EU/EEA countries, it has been hypothesised that the proportion of clinically severe cases could be increasing owing to the increasing proportion of susceptible individuals in older age-groups at risk of severe disease (4).

Since the 1990's, safe and effective hepatitis A vaccines are available. Vaccination provides immunity for many years, possibly for life. The World Health organization (WHO) recommends universal hepatitis A vaccination of toddlers in intermediate HAV endemicity areas and vaccination of risk groups in very low and low endemicity areas. Risk groups include those at increased risk of infection like travellers to HAV endemic areas and men who have sex with men (MSM), amongst others (5).

ECDC and most EU/EEA countries echo these recommendations. However, in the last 20 years large outbreaks associated with these groups have been often reported. Furthermore, an increasing number of foodborne infections and outbreaks associated with contaminated food have been reported making of hepatitis A a threat to public health in the EU/EEA (4, 6).

2 LITERATURE REVIEW

2.1 Historical background and evolutionary biology

Epidemic jaundice is reported in Mesopotamian (Talmud), Mediterranean (Hippocratic corpus), and Chinese literature as early as the fifth century BC (7, 8). Epidemics of jaundice were reported in several European countries during the 18th and 19th centuries, affecting both children and soldiers, occasionally in large numbers (8). In the late 1800s Bamberger and Virchow advanced the concept of "catarrhal jaundice" to describe epidemic jaundice (9). This concept, however, included several forms of both non-infectious and infectious hepatitis. During the rest of the 19th century and the early decades of the 20th century, Lürmann, Cockayne and others recognised the infectious nature of what they called "icterus epidemics" and "infective jaundice epidemics" but made small progress in characterising the related aetiological agents, which most likely included not only HAV but also other hepatitis viruses and bacteria like *Leptospira* (8, 10).

The hypothesis of two infectious types of hepatitis was first advanced in the 1940s by MacCallum, who reported on two separate types of infectious hepatitis associated with an "icterogenic agent". The first was generally affecting children and often associated with consumption of water or food contaminated by human faeces. The second was associated with injection of human blood components, as observed in soldiers deployed during the second world war, who developed hepatitis and jaundice after injection of batches of yellow fever vaccine prepared using human serum (11-13).

In 1967 Krugman, Giles and Hammond published the results of an ethically controversial study where the study participants were school children with disabilities deliberately infected for experimental purposes. The study described the transmission and features of two types of infectious hepatitis, "MS-1" and "MS-2" (14). The causative virus of the first type of hepatitis, HAV, was first detected in 1973 by Feinstone using electron microscopy, making it possible to draw a clear diagnostic difference between HAV and HBV (15). In 1975, Feinstone's team also first reported an analysis of HAV antigen shedding patterns in stools during the different clinical phases of the disease, confirming that electron microscopy was a reliable diagnostic tool (16).

In 1975, Maynard demonstrated through immune electron microscopy the experimental infection of non-human primates (marmosets, monkeys and chimpanzees), providing the scientific community with an experimental model for HAV infection (17-19). HAV research in non-human primates opened the path to *in vitro* and *in vivo* experiments leading to HAV isolation in cell culture and to studies to understand the HAV pathogenesis and immune response (20, 21).

From 1978, live attenuated and formalin-inactivated hepatitis A vaccines were experimented in marmosets, chimpanzees and humans (22). Both formulations showed high tolerability, efficacy and immunogenicity in animal models and in humans, being then licensed in the early 1990s (7, 20, 23).

The full sequence of nucleotides of the HAV genome was first determined in 1985 (24). In 1989, Brown et al. showed that the VP1/2A fragment was the HAV genomic region with the highest genetic variability (25). This genomic region remains the main focus of sequencing efforts and related phylogenetic analysis today.

2.2 Hepatitis A virus

HAV is a hepatotropic virus of the family *Picornaviridae*, genus *Hepatovirus*, containing a positive sense, linear, single strand of RNA. The genome is relatively short, approximately 7500 nucleotides long, with one single open reading frame divided into three functional regions: P1, P2 and P3. P1 translates a large polyprotein (the viral capsid protein) consisting of four regions: VP1, VP2, VP3 and VP4. P2 and P3 express the regions 2A–2C and 3A–3D, which are non-structural polyproteins required for virus replication (3, 26).

In 2018, Lemon et al. reported that the VP2 protein is folded in a very different way from other viruses of the *Picornaviridae* family. Such characteristic has been observed only in primitive picorna-like viruses of insects and could testify to the very ancient origin of HAV. The same feature could also participate in the mechanism providing HAV with resistance to temperature and acid inactivation, which gives HAV the ability to survive in the environment as well as in food, sewage and human skin. This offers advantages to the virus' faecal-oral transmission (3, 27-29).

Infectious HAV exists in both non-enveloped (naked) and enveloped (defined as quasi-enveloped) forms. As proven by recent studies, this very unusual property is only identified in the hepatitis E virus. Quasi-enveloped virions (50–110 nanometre in diameter) are found in the blood of infected individuals. The envelope contains a capsid, which in turn contains the viral RNA. The envelope protects the virions from detection by B cells and from neutralising antibodies (30). The naked virions are small and round (27 nanometre in diameter) and have an icosahedral protein capsid containing the genome (15, 31). The lack of envelope makes the virion resistant to dry conditions and stable in the environment, facilitating the spread to naïve hosts (32).

All HAVs infecting humans are part of the same serotype due to the virus' highly conserved antigenic structure (33, 34). Based on some modest genetic diversity in the VP1 region and the VP1/2A junction, HAV strains are classified in five genotypes (35): genotype I to III viruses infect humans, while genotypes IV to VI are of simian origin (26, 36). The two groups of genotypes present significant differences in the P1 fragment and in the junction VP3/VP1 (36, 37). Each I-III genotype is subdivided into sub-genotypes (A and B) with a genetic variation between sub-genotype of >7% (38) (Figure 2.2.1). The origin of each sub-genotype has been associated with large geographical areas where the sub-genotype has traditionally circulated. This approach has obvious limitations due to global imbalances in sampling strategies and complex patterns of HAV circulation, particularly during the last two decades. Nonetheless, it has been observed that the sub-genotype IA is the most prevalent in Africa, the Americas, Asia and Europe (37, 39). Sub-genotype IB circulates in Northern Africa and Middle East countries, in addition to South Africa and parts of Brazil (37). Interestingly, sub-genotype IB has become the most prevalent sub-genotype in the USA after 2015, replacing sub-genotype IA (37, 40). Sub-genotype IIA is the most prevalent only in some areas of western Africa; whilst sub-genotype IIB has been very rarely reported and appears difficult to describe (41). Sub-genotype IIIA has been reported to circulate in central and south Asia (37). Sub-genotype IIIB has been reported to have circulated in Japan long ago and on very few occasions; since Japan is witnessing an extremely low HAV endemicity, sub-genotype IIIB may have been eliminated (39, 42, 43).

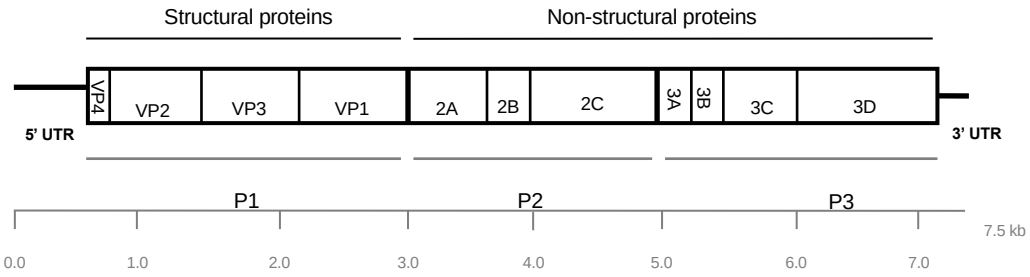


Figure 2.2.1. Hepatitis A virus genotype genome regions. Source Enkirch T. (2019) (26)

Few studies suggest that infection with sub-genotype IB and IIIA is associated with more severe disease (44, 45); however, the mechanism behind the possible increased virulence of these sub-genotypes has not been understood (46, 47).

Different HAV sub-genotypes can simultaneously infect a susceptible individual, but it is unclear what such co-infections imply in terms of viral natural history, pathogenesis and genetic recombination (37).

2.3 Transmission

Most often, transmission occurs via ingestion of the virus through faecal–oral transmission, either by consumption of contaminated food or water or by person-to-person contact (48). Food and waterborne transmission, either direct or mediated by food-handlers, is common due to the virus' capacity to resist acidification and temperature stress (32). Person-to-person transmission entails a high degree of close contact and often occurs in the household or in schools; it also includes sexual transmission, which is mainly associated with high-risk sexual behaviours (49, 50). Parenteral transmission, through infected syringes, blood components or other substances of human origin, has been rarely reported, even though it requires a much lower infectious dose than oral transmission (37, 51).

2.4 Pathophysiology and immune response

HAV has an unusual and still not very well understood life cycle. After ingestion, HAV enters the gut mucosa as a naked virus; it then reaches the liver, most likely through the portal circulation, as a quasi-enveloped virus. This is possibly thanks to a first, moderate, extra-hepatic replication occurring in the intestinal epithelium. Once in the liver, primary replication occurs in the hepatocytes (52, 53).

The HAV-host interactions are not completely understood. Until recently, HAV entry in epithelial cells and hepatocytes (both in the enveloped and naked form) had been thought to happen through the calcium-dependent cellular receptor TIM1, also named HAVCR1 (54, 55); however, it is now proven that TIM1 is not essential and that the virus can use different entry pathways as long as cellular gangliosides are implicated to bind the capsid and free the viral genome inside the cell (47, 56, 57).

After intracellular replication, HAV is released from the infected hepatocyte in the biliary tract as its quasi-enveloped form in a mostly non-cytopathic fashion, through a non-lytic, immune-mediated inflammatory process (58, 59). Due to the acidification of the bile, the virus' envelope is lost, and the naked virus is released in the intestine, from where it is shed in stools (56).

HAV infection and immune response in non-human primates, particularly in chimpanzees, accurately models the mechanisms in humans (60, 61). Infection dynamics have been found to diverge in blood,

stool and liver. HAV shedding in stools is identifiable about a week after intravenous inoculation and throughout the following five weeks. Viremia appears at about the same time and continues for about two to three weeks. HAV takes longer to be detected in the hepatocyte (after about three weeks from inoculation), where it persists for longer, with HVA RNA being identifiable for many months after inoculation. The presence of HAV RNA does not imply ongoing virus replication, although this could explain the clinical manifestation of relapsing hepatitis and the persistence of HAV antibodies (21, 53, 62, 63). In the same animal models, clinical symptoms and hepatitis were developed after three to five weeks from inoculation, whilst anti-HAV specific antibodies (IgM) appeared after three to four weeks, probably playing an essential role in clearing the virus from blood (64).

HAV replication is not considered directly responsible for liver damage since both maximal viraemia and viral shedding occur one to three weeks before the alanine aminotransferase (ALT) peak and the IgM peak (65, 66). Although a clear explanation is lacking, liver damage appears to be due to the host immune response and the cytotoxic effect of T-cells; whilst its extent, thus the degree of disease severity, appears associated with the polymorphism of the gene encoding the receptor TIM1 (46, 67, 68).

Infection can be symptomatic or asymptomatic. Symptomatic infection is defined by elevated serum bilirubin and ALT levels, which can be preceded by mild symptoms, including jaundice (3). In asymptomatic or mild HAV infections, serum bilirubin and ALT levels are higher than baseline but still much lower than in patients with acute hepatitis A. ALT levels, which are markers of liver damage, can remain elevated for long periods following both symptomatic and asymptomatic infection (66, 69).

HAV appears to have the capacity to suppress the innate host immunity by suppressing hepatic interferon responses (21). Humoral immune response to HAV appears only after replication in the hepatocyte. Anti-HAV IgM can be identified a few days before the onset of symptoms, at the start of the increase in ALT, whilst anti-HAV IgG and IgA appear a few days later. IgM and IgG are detectable in asymptomatic patients about three and four weeks, respectively, after infection. IgM are detectable for about four months, whilst IgG, which dominates the immunological response, persist for many years, possibly lifelong, and protects from reinfection (4, 66, 70).

2.5 Clinical presentation

For most children ≤5 years of age HAV infection is asymptomatic. In older children and young adults, the infection is also often asymptomatic or very mild, with the frequency of clinical manifestations and their severity increasing with age. Older adults often experience a severe disease and may need hospitalisation (3).

In symptomatic patients, after an incubation period of about four weeks, ranging from two to seven weeks, non-specific (fatigue, vomiting, anorexia, diarrhoea, fever) and specific (dark urine, clay-coloured urine and jaundice) symptoms appear. As mentioned above, virtually all patients experience a remarkable increase in ALT and bilirubin serum levels, whereas jaundice occurs in about 10% of children ≤5 years of age and 70% of adult patients (71). Although rare, a wide range of atypical and extra-hepatic manifestations have been reported, including prolonged cholestatic hepatitis, acute kidney injury, vasculitis, pancreatitis, meningo-encephalitis and Guillain–Barré syndrome (71).

Acute liver failure (fulminant hepatitis) is a very severe complication of hepatitis A occurring in <1% of patients. Age, chronic liver disease, concurrent infection with other viruses, including other hepatitis viruses and, possibly, human immunodeficiency virus (HIV), are all associated with an increased risk

of acute liver failure (72). Genetic variations in the host may also play a role (67, 68). In India, the Republic of Korea, Argentina, Brazil and other South American countries, HAV infection is one of the main causes of fulminant hepatitis in children (73-75). Acute liver failure may resolve spontaneously or require liver transplantation; when liver transplant is not an available option, paediatric and adult patients in need of it experience a case-fatality ≥75% (76, 77).

Pregnant women, particularly if infected in the third trimester of pregnancy, are at increased risk of maternal complications and pre-term labour. Mother and foetal outcomes are generally benign. Mother-to-child transmission has not been reported (71). Children born from seropositive mothers have been transferred anti-HAV antibodies, which persist for six months to one year after birth (78).

Estimates of the hepatitis A case-fatality range from 0.1% in children <15 years of age to about 5% in adults >50 years of age (69, 79, 80). However, recent estimates are lacking, and these estimates possibly provide an overestimation of the current hepatitis A case-fatality (4).

Ninety-nine per cent of the patients recover within two months from the onset of symptoms. Three to 20% of patients experience relapsing symptoms. Relapsing hepatitis is characterised by the reappearance of clinical symptoms, a new increase in ALT levels, viraemia and viral shedding in stools after resolution of the first clinical episode. Relapse symptoms may re-appear within six months after onset or longer and are generally milder than those associated with the primary disease (4, 63). HAV infection confers lifelong immunity and does not cause chronic infection or chronic liver disease (81).

2.6 Diagnosis and diagnostics

Hepatitis A clinical diagnosis is based on clinical symptoms and history of risk factors for HAV infection. However, since hepatitis A symptoms are undistinguishable from other viral hepatitis, laboratory confirmation is required (82).

Traditionally, laboratory diagnosis is based on serological detection of anti-HAV IgM in serum or plasma samples through enzyme immunoassay or several other methods. Anti-HAV IgG testing may also be performed to improve diagnostic accuracy. Such techniques continue to be valid in absence of routine RNA testing (83-86). Anti-HAV IgM testing has some limitations due to the low anti-HAV IgM specificity, which challenges the interpretation of equivocal or low-level results in samples from asymptomatic or older individuals (87). Anti-HAV IgG testing, along with the patient's clinical, biochemical and vaccination information, can be essential to support result interpretation (4, 70).

Detection of HAV RNA by RT-PCR in serum, stools and saliva samples has proven to be effective in diagnosing and monitoring HAV infection, particularly at earlier stages. RT-PCR is also widely used to detect HAV in environmental samples (85, 86).

HAV characterisation and phylogenetic analysis is performed by nucleic acid sequencing on purified PCR products obtained from serum (86). Sequencing data matched with epidemiological information from the cases provide strain characterisation, geographical analysis and assistance to public health investigations (26, 88). Globally, different sequencing protocols target a range of HAV genomic regions. Traditionally, the VP1/2A fragment has been the main targeted genomic region for sequencing thanks to its relatively high genetic variability (89). However, over the last three decades many different protocols have targeted different genomic regions, resulting in a large number of scattered fragments, different in genomic position and length, and only partially, or not at all, overlapping (40, 90, 91). Such diversity limits the use of sequences available in public repositories as only a part of the sequences'

genetic information can be compared (39). Since 2014, the European Centre for Disease Prevention and Control (ECDC) promotes the use of the HAVNet protocol or other protocols targeting an overlapping genomic region covering a 460 nt long fragment in the VP1/2A-region (26, 92, 93).

Genomic sequencing is witnessing rapid progress in speed and sample turn-around as well as a strong reduction in technological costs. Complete sequences offer the maximum genetic resolution for HAV strain characterisation. Thus, a move towards the sequencing of complete genomes has been advocated (39).

The history of natural infection or immunisation (thus immunity to HAV) is tested by detecting anti-HAV IgG in serum, plasma or oral fluids. In the absence of health records testifying history of infection or vaccination, serology can help decide on the management of contacts of cases and, possibly, support investigation of clusters defining the direction of infection in household secondary transmissions, particularly when children are implicated (94, 95). Highly sensitive immune assays, able to distinguish between immunity from natural infection or vaccination, exist but are not commercially available yet. Once accessible, they will be useful to support interpretation of seroprevalence studies by discerning between immunity from HAV infection or vaccination, so to clarify the impact of vaccination on population immunity in the medium and long term, and guide vaccination policies (4, 96-98).

2.7 Treatment

Treatment for hepatitis A is supportive as there is no specific therapy. WHO does not recommend the use of unnecessary medications, including acetaminophen or antiemetics, unless in case of severe vomiting. Hospitalisation should also be avoided in the absence of acute liver failure or other severe complications (99). Due to the risk of acute kidney injury, the renal function should be checked (71). In case of fulminant hepatitis, liver transplant can strongly reduce the risk of death in paediatric and adult patients requiring transplantation (76, 77, 100).

2.8 Hepatitis A virus endemicity and epidemiology

WHO defines endemicity as “high”, “intermediate”, “low” and “very low” on the basis of seropositivity to anti-HAV IgG in the population at age 10, 15 and 30 years (5).

In high endemicity countries, >90% of children are seropositive by age 10 years. The virus widely circulates in the community due to poor access to safe drinking water and food, and sub-optimal sanitation. Infection happens at young age and most cases are asymptomatic. Overall, this results in a relatively small number of reported hepatitis A cases, both because most infections are asymptomatic and because of contextual surveillance system limitations (101, 102). Countries in this category are low-income Sub-Saharan, northern African, Middle Eastern and southern Asian countries (5).

In many countries, socio-economic and human development progresses, both at the individual and community level, have rapidly increased and are reflected in improved housing, food safety, sanitation, and access to clean water and vaccination. Such countries are witnessing an epidemiological transition, moving from high or intermediate endemicity to the lower endemicity level (101).

In intermediate endemicity countries, ≥50% of children by age 15 years, with <90% by age 10 years are seropositive. In these settings, HAV circulation gives rise to sometimes very large hepatitis A outbreaks hitting pockets of susceptible adults who escaped infection at young age. Since adolescents and young adults more frequently have severe clinical manifestations than children, not only the number of

symptomatic cases, but also the number of clinically severe infections is much higher than in other endemicity settings. Some middle-income countries in northern Africa, Middle East, southeast Asia, central and south America, and eastern Europe are part of this category (5).

In low endemicity countries, ≥50% of adults by age 30 years and <50% of children by age 15 are seropositive. The level of virus circulation is low, except for those groups at increased risk of infection. Most cases are reported in older children and young adults (1). Some middle-income countries in central and south America, east Asia and eastern Europe are part of this category (5).

In very low endemicity countries, <50% of adults are seropositive by age 30 years. The virus is almost not circulating, and the risk of infection is very low for those susceptible. The annual hepatitis A incidence in these countries can be as low as <1 case per 100,000 population (see Study 5). Most high-income countries in north America, east Asia, Europe and Oceania are part of this category (5).

In very low and low endemicity countries, most cases are associated either with travel to endemic countries or with foodborne infection in the country of origin. However, population groups sustaining a continuous (or quasi-continuous) HAV circulation remain. Those are mostly ethnic minorities, people experiencing homelessness and sexual networks of individuals engaging in high-risk sexual behaviours. People participating to these groups are also in contact with the rest of the population, which is mostly susceptible to HAV infection, giving rise to spill-over events in the general community, mostly in household, nursery and school contacts, and via infected food-handlers (103).

2.9 Burden of hepatitis A

The Global Burden of Disease Study 2019 rates hepatitis A as the most common acute viral infection at the global level, with 159M new infections and about 4,000 deaths in 2019, with sub-Saharan Africa and Asia bearing the largest part of the hepatitis A mortality (1).

Recent European studies on the burden of hepatitis A are available for Denmark (1) and the Netherlands (2). The Danish study, after accounting for underreporting, estimated 126 hepatitis A cases and one death in 2019, with a health burden of nine disability-adjusted life years (DALYs) and a financial burden of 1M euro, with 42% of the cases attributed to foodborne transmission (104). The first Dutch study estimated 612 hepatitis A cases and two deaths in 2011, with a health burden of 98 DALYs per year and a financial burden of 0.9M euro (105). The second Dutch study estimated 900 hepatitis A cases and three deaths in 2018, with a health burden of 100 DALYs per year and a financial burden of 1.5M euro, with 69% of the cases attributed to foodborne transmission (106).

2.10 Risk groups

Hepatitis A risk groups can be divided into those groups at increased risk of infection and those at increased risk of a clinically severe disease (i.e. hospitalisation, prolonged disease, liver failure) (5).

In all endemicity settings, susceptible individuals not having access to robust public health infrastructures (i.e. lacking access to safe water, sanitation and adequate housing) and in contact with an infected person are at increased risk of infection. In very low and low endemicity settings, groups at increased risk of infection include travellers to higher endemicity areas, MSM, PWUD, people suffering of homelessness, ethnic minorities, laboratory staff dealing with HAV, parents of newly adopted children, staff of children nurseries, workers in contact with sewage waters and frequent recipients of blood products (3).

Groups at increased risk of severe outcome include older adults and patients with underlying chronic liver disease (107). It is debated whether HIV patients are also part of this group, as their increased probability of a severe outcome appears associated with coexisting liver conditions (e.g., coinfection with HBV and HCV, liver damage from antiviral treatment or alcohol abuse) and disappears in patients compliant to antiretroviral treatment (108, 109).

2.11 Hepatitis A outbreaks

The largest outbreak reported in literature occurred in Shanghai, China, in January and February 1988. Health authorities reported 310,000 cases of hepatitis A, including 47 deaths. About 90% of the 8,000 hospitalised patients were aged between 20 and 40 years (110, 111). The outbreak, associated with consumption of contaminated clams, magnifies the characteristics of foodborne outbreaks, where the frequency of new cases rapidly declines after the withdraw of the contaminated food item. It also magnifies the characteristics of outbreaks in large and densely populated areas at intermediate endemicity, where very few older adults are infected as they are virtually all immune.

In very low and low endemicity settings, foodborne outbreaks are often reported, sometimes involving even hundreds or thousands of cases, as in Michigan and Main, USA, in 1998 and in 13 EU/EEA countries in the 2013-2014 outbreak (112, 113). The European foodborne outbreak differed from the Shanghai outbreak and, as expected in very low endemicity settings, affected older adults and had a much higher proportion of hospitalised patients (113). Several contaminated food items have been involved in hepatitis A outbreaks as vehicles of foodborne infections: shellfish, berries, pomegranates, dates, other fruit and vegetables, often imported as frozen product and consumed without heat-treatment (114-116). Foodborne outbreaks associated with infected food handlers are also common, although most times these are smaller in size and tend to be geographically confined (114).

Waterborne outbreaks of hepatitis A are generally associated with waters inadequately treated and are more common in higher endemicity than in very low or low endemicity settings (117).

Outbreaks associated with high-risk sexual behaviours, often involving MSM networks, are commonly reported, particularly in very low and low endemicity settings. The largest outbreak of this kind was reported in the EU/EEA in 2016-2018. This outbreak, driven by person-to-person transmission, mostly affected MSM and was amplified by spillovers into other groups at increased risk of infection as well as the general community (103). International travel played a key role in the 2016-2018 outbreak making of it a global event with cases reported in the Americas, Asia, Europe and Oceania (50, 118). A previous large hepatitis A outbreak driven by person-to-person transmission and affecting different networks (MSM, PWUD, ethnic minorities) in several EU countries was reported in 2008-2009 (119-124).

Outbreaks in travellers from low endemicity to higher endemicity countries are also common in the literature. In such outbreaks, travellers are generally infected through foodborne transmission after exposure to contaminated food items, sometimes in large numbers, or through person-to-person transmission because of unsound hygiene practices (125-127).

Recent outbreaks have had a longer duration. This is possibly due to i) the increased use of genome sequencing, which allows to link sporadic cases or geographically separated outbreaks to the same vehicle or transmission event; ii) increased globalisation and international trade and travelling; iii) societal changes due to apparently independent public health emergencies (26, 101, 127, 128). The USA is witnessing a prolonged multistate hepatitis A epidemic ongoing since 2015. It mostly involves people

experiencing homelessness and PWUD. After two decades of steeply decreasing trends, US hepatitis A notification rates have increased >10 times from 2015 to 2019 and the main circulating sub-genotype has shifted from IA to IB, which is associated with the current epidemic. Such prolonged event provides an alert on how specific population groups, if neglected, can sustain intense HAV transmission for many years in very low endemicity settings implementing universal hepatitis A vaccination of toddlers (66, 128).

2.12 Active and passive immunisation

Hepatitis A is a disease preventable both through active and passive immunisation. The first is attained through vaccination, the second using human immune serum globulin (Ig).

Hepatitis A vaccines are available either as formaldehyde-inactivated, used globally, or as live attenuated, mostly used in China and, to a lesser extent, in other Asian countries and Guatemala (4, 129, 130). All vaccines are licensed for use in children aged 1 year or older (99).

The inactivated vaccine is licensed for intra-muscular administration in a two doses schedule with at least 6 months interval between the first and the second dose. It is extremely immunogenic, eliciting quasi-100% seroconversion in children and healthy adults <60 years of age. In these recipients, inactivated vaccines lead to rapid seroconversion (within 2-4 weeks after first dose) and protection lasting for decades, if not for life (129, 131). Maternal antibodies, HIV status, smoking and increasing age, weight and body mass index reduce the immune response, whereas being of the female gender increases it (129). Over the last three decades the vaccine has proven to be safe and well tolerated, making it a safe and effective option for pregnant women at risk of HAV infection and immunosuppressed patients (4, 5, 132, 133). Inactivated hepatitis A vaccines are made available as a single vaccine formulation or in combination with other vaccines (e.g., hepatitis B or typhoid) by many manufacturers. All inactivated hepatitis A vaccine are interchangeable (4, 5).

The live attenuated vaccine is used in a single dose schedule given subcutaneously. Seroconversion is slower and less pronounced than for hepatitis A inactivated vaccines. Seroconversion rates in children participating to six controlled clinical trials conducted in China range from 62 to 97% at different points in time. About 25% of vaccinated individuals shed the vaccine strain in stools for about a month after vaccination. After shedding the vaccine strain appears stable and neither reversion to virulence, nor seroconversion events in contacts of vaccinated individuals have been reported. The vaccine is considered safe and able to provide immunity for many years; however, as per other live attenuated vaccines, it should not be administered to pregnant women or immunocompromised patients (4, 5, 134-137).

Ig, which contain human anti-HAV antibodies, have shown 80 to 90% effectiveness in protecting from liver disease when administered within 2 weeks from exposure. Ig are protective within hours from their intramuscular administration. Their effect lasts 2 to 5 months, after which the recipient's immunity is lost. Ig are safe but should not be administrated to patients with IgA deficiency or individuals receiving live attenuated vaccines (including hepatitis A live attenuated vaccines). Ig have long been used to prevent infection, but over the last three decades their use has been strongly reduced due to a number of factors. These include the cost, the availability of cheaper vaccines providing immunity rapidly and for decades, their limited stock and the decreased availability of human plasma pools containing enough anti-HAV IgG. They are indicated for post-exposure prophylaxis (PeP), as soon as possible after exposure and no longer than 14 days (4, 138).

2.13 Immunisation strategies

WHO position: in the 2012 Position Paper on Hepatitis A Vaccines, WHO set recommendations on immunisation strategies based on country endemicity levels, as these provide an efficient rapid assessment tool to orient prevention policies (5). In high endemicity countries WHO does not recommend large scale hepatitis A vaccination. In intermediate endemicity countries, WHO recommends universal hepatitis A vaccination of toddlers; such a policy should be pondered after economic evaluations and should be implemented along with improvements in hygiene and sanitation, and build-up of capacities for surveillance, outbreak response and monitoring of vaccination impact. In low and very low endemicity countries, WHO recommends targeted vaccination of individuals at increased risk of infection or at increased risk of severe outcome, referring to travellers to endemic areas, MSM, PWUD, workers in contact with human primates, patients requiring life-long treatment with blood products or those with chronic liver disease (5). In the 2019 Immunological Basis for Immunization Series on Hepatitis A, WHO recommends vaccination to additional groups including food-handlers, day-care centre staff, garbage and sewage workers, people experiencing homelessness, parents of adoptees born in HAV endemic countries, children of refugees and migrants from HAV-endemic countries (4). An updated WHO position paper on hepatitis A vaccines is expected to be published while this thesis is in printing.

Universal vaccination of toddlers: over the last 25 years, a number of countries or subnational regions with high hepatitis A incidence have implemented the WHO recommendation of universal vaccination of toddlers. In Israel, Panama, Uruguay, U.S.A, amongst others, this policy has been implemented using two doses of hepatitis A inactivated vaccine (4, 129). In Argentina, Brazil, Nicaragua and the Republic of Korea, amongst others, universal vaccination of toddlers has been implemented using a single dose schedule of inactivated hepatitis A vaccine, whereas in China the same policy was implemented using a single dose schedule of live attenuated hepatitis A vaccine (4, 129). Following the roll-out of the vaccination programmes, studies performed in these countries showed a decline in the incidence of hepatitis A and/or of fulminant hepatitis, and/or in the frequency of hospitalisations, liver transplantations and mortality associated with viral hepatitis (4, 129).

Single dose schedule of hepatitis A inactivated vaccines: the use of a single dose schedule of inactivated vaccines, instead of the two doses schedule as per the vaccine license, is driven by the experience that immunogenicity after a single dose is almost as high as with two doses, whereas the economical and logistical costs of the vaccination programme are much lower (4, 139-141). Modelling studies on immunogenicity predict 30 years of protection for about 90% of children primed with one dose of hepatitis A inactivated vaccine (142). Whether exposure to circulating HAV viruses can provide "natural boosting" of the immunity of those vaccinated with a single dose schedule remains unclear and possibly unlikely (129).

Additional dose: it is debated whether elderly people or immunocompromised patients (e.g., HIV positive or chronic liver disease patients) may need additional vaccine doses. This would include both "double priming" (e.g., two doses with a 4-week interval, in addition to a third dose after ≥6 months) to elicit a rapid immune response, and a "late booster" (i.e. the administration of an additional dose after 10 years from the regular 2-doses vaccination schedule) to ensure long lasting immunity. Regarding the possibility of administering a third dose to healthy people after 20 or 30 years from the second dose of an inactivated vaccine, such a late booster dose does not appear necessary (143).

Lastly, there is currently no evidence on the need to vaccinate with a late booster dose those individuals that were vaccinated with a single dose schedule of inactivated or live attenuated vaccine (4, 129).

Post-exposure prophylaxis (PeP): PeP is indicated in susceptible individuals exposed to HAV (e.g., contacts of HAV infected individuals in households, work environments, child day cares, particularly during outbreaks). It is administered either as an inactivated vaccine or as Ig. The use of an inactivated vaccine is most often preferred since it provides longer protection, is more cost-effective and logistically simpler to procure. Both vaccine and Ig show high protection performance. Ig is also considered able to reduce the severity of the symptoms even when administered after two weeks from exposure (144-146). Ig indications in infants are not completely consistent in different countries: for instance, in Canada and the USA, Ig are indicated in infants <6 or <12 months of age, respectively, whereas in the UK Ig is not indicated in infants <12 months. Ig are indicated for those allergic to the vaccine or its components. Administration of both inactivated vaccine and Ig (in different parts of the body) should be given to immunocompromised patients, including those with chronic liver disease, to people aged ≥60 years and to those at risk of a severe outcome after HAV infection (4, 83, 147-149).

Outbreak control: in low and very low endemicity settings, hepatitis A outbreaks involving well-defined communities can be controlled through one dose of inactivated vaccine (e.g., in educational and work environments, ethnic minority communities, larger mixed communities like villages or small towns). Ig can also be used, but the vaccine is the most effective option for most members of such community (150-153). Unless rapidly implemented, vaccination and Ig may not prevent secondary cases, but are effective in stopping tertiary cases (83). Selective vaccination of susceptible children and close contacts of cases can also be implemented if achieving rapid, high coverage of the entire population is not feasible (83). In any setting, outbreak control efforts should also improve sanitation, provide health and hygiene education and include social mobilisation (5, 83). There is no evidence of the effectiveness of using one dose of inactivated vaccine to control outbreaks taking place in large and not easily definable communities. However, some indirect evidence suggests that large widespread outbreaks can be controlled, or at least slowed and reduced in size, through vaccination, as suggested by the largest hepatitis A outbreak disproportionally affecting MSM ever reported in Europe (see Study 3) and its temporal association with the unavailability of hepatitis A vaccine due to a global shortage. In 2017, because of the limited supply of vaccine and the extent of the outbreak, ECDC and some EU Member States affected by the shortage recommended temporary options including the prioritisation and reinforcement of the vaccination in MSM to maximise the available stocks. Such recommendations included the off-label use of paediatric vaccines for adults, re-prioritisation of the groups to be vaccinated, delay of second doses (except for immunocompromised patients) and the use of serological testing before vaccination (50, 83, 154).

2.14 Vaccination policies in the EU/EEA

Greece is the only EU/EEA country implementing universal vaccination of toddlers at the national level, a policy that began in 2008. Children older than 12 months are vaccinated with a two-dose schedule at an interval of 6 months; the cost of the vaccination is publicly reimbursed. As of 2015, the coverage of one dose vaccination in children was reported to be about 80%. In the same year, Mellou et al. estimated that the annual cost of the programme was about 8 million euros and reported that an alternative prevention plan offering vaccination to selected risk group, health education to the general population and enhanced food safety could have been more cost-effective (155).

At the subnational level, Apulia, in Italy, since 1998 offers free-of-charge hepatitis A vaccination to toddlers 15-18 months old and, as a catch-up campaign, to children 12 years old. The schedule includes two doses with an interval of 6 months. As of 2018, the two doses-vaccination coverage reported in children born in 2015 was 74% and in those born in 2014 was 78% (156).

From 1998 to 2013, the region of Catalonia, Spain, implemented a schedule with three doses of hepatitis A + B vaccine given within 6 months at the age of 12 years; from 2014 to 2017 the schedule has been of three doses of monovalent hepatitis A vaccine at age 12 months, six and 12 years; and since 2018 the schedule is of two doses at 12 months and six years of age (157). In Ceuta and Melilla, since 2000 two doses of hepatitis A vaccine are given at 15 and 24 months of age (158).

In Germany, the federal state of Saxony recommends either a full schedule of hepatitis A+B vaccine or a single hepatitis A vaccination for children between 2 and 17 years of age (159).

In all other EU/EEA countries and subnational areas, vaccination of selected groups at either increased risk of HAV infection or increase risk of severe outcome is recommended. In 2015, ECDC mapped hepatitis A vaccination policies in EU/EEA countries and identified substantial differences in the definition of at-risk groups and in the ways to reach them out (160).

Only inactivated vaccines are licensed in the EU/EEA (4).

2.15 Cost-effectiveness studies in the EU/EEA

Interpreting cost-effectiveness analyses older than 20 years is challenging since hepatitis A incidence, HAV endemicity, policy cost and the use of prevention tools (e.g., hepatitis A vaccine versus Ig) may have significantly changed. Here we look at cost-effectiveness analyses performed in EU/EEA countries and reported in English literature after 2000.

Analyses exploring the cost-effectiveness of universal vaccination of toddlers were performed in Bulgaria in 2014, in the Netherlands in 2012, in Italy in 2003 and in Germany in 2001. The studies performed in Bulgaria and in Italy identified that universal vaccination would be cost-effective only during high-incidence years (161, 162), whereas the Dutch and German studies found universal vaccination of toddlers not to be cost-effective (163, 164).

Analyses exploring the cost-effectiveness of vaccination policies targeting specific population groups were performed in Belgium in 2012, in the Netherlands in 2004 and 2012, and in Italy in 2003. The Belgian study explored the cost-effectiveness of vaccinating adults and found that such approach would not be cost-effective in a low endemicity country (165). The two Dutch studies looked at two different approaches: in the 2004 study the authors assessed whether vaccinating children of ethnic minorities, at increased risk of infection due to travelling to high or intermediate endemicity countries to visit friends and families, could be cost-effective; whereas in the 2012 study the cost-effectiveness of vaccinating risk group (*status quo* policy) was explored. Both studies found that such policies would be cost-effective (164, 166). The Italian study assessed that vaccinating close contacts of cases would be cost-effective (161).

A British study from 2018 explored whether it was more cost-effective vaccinating MSM with or without offering prior to vaccination a screening test of their seropositivity. The study results indicated that vaccinating only seronegative MSM was not cost-saving and the study investigators recommended vaccinating MSM without prior testing to improve vaccine uptake (167).

2.16 Hepatitis A surveillance

As for most infectious diseases, hepatitis A surveillance can be broken up into indicator-based surveillance (IBS), fed by a regular flow of hepatitis A notifications within local, national and international surveillance systems, and event-based surveillance (EBS), collecting unstructured information on specific events. On one hand, a strong IBS framework is crucial to assess disease trends, identify risk factors, monitor efforts in prevention and control, and plan policy options and investments. On the other hand, EBS provides rapid information and is essential for early detection of outbreaks, particularly for outbreak-prone pathogens like HAV (168).

Hepatitis A surveillance complies with the International Health Regulation and is part of the EU surveillance framework (169, 170).

2.17 Indicator-based surveillance in the EU/EEA

Hepatitis A is a notifiable disease in all EU/EEA countries. As for most other diseases under surveillance, it entails data gathering, data analysis and data communication. Hepatitis A surveillance strategies vary within the EU/EEA countries: HAV notifications can be reported by clinicians, public health laboratories, and/or hospitals depending on the way each national system is structured. Notifications fitting the surveillance case definition are reported to the health service at the subnational and national level, and subsequently to the EU/EEA level through an annual data call to The European Surveillance System (TESSy); after data validation, TESSy data are made available to the public in the ECDC Atlas of Infectious Diseases (2, 171).

IBS in the EU/EEA aims at i) monitoring trends in hepatitis A over time and across countries; ii) identify groups at increased risk of infection and at increased risk of severe outcome; iii) retrospectively assess large hepatitis A outbreaks; iv) monitor national and European surveillance to improve it; and v) contributing to the assessment of the burden of hepatitis A (172).

The majority of the EU/EEA countries use the EU case definitions set in the European Commission Implementing Decision (EU) 2018/945 or previous versions (170, 171). Such definitions set clinical, microbiological and epidemiological criteria to define cases (Figure 2.17.1), also in line with the WHO nomenclature and the 10th Revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) (173). Since hepatitis A clinical symptoms are not specific, a laboratory confirmation of the infection is essential to define an EU/EEA confirmed case and may be sufficient for confirmation if the country does not collect/report information on clinical symptoms. Out of the EU/EEA, due to the diverse availability of diagnostics, an epidemiological link to a confirmed case can also be sufficient to define a confirmed case; however, in the EU case definition, such a link defines only a probable case (170, 173).

ACUTE HEPATITIS A

Clinical Criteria

Any person with a discrete onset of symptoms (for example, fatigue, abdominal pain, loss of appetite, intermittent nausea and vomiting)
AND
At least one of the following three:

- Fever
- Jaundice
- Elevated serum aminotransferase levels

Laboratory Criteria

At least one of the following three:

- Detection of hepatitis A virus nucleic acid in serum or stool
- Hepatitis A virus specific antibody response
- Detection of hepatitis A virus antigen in stool

Epidemiological Criteria

At least one of the following four:

- Human to human transmission
- Exposure to a common source
- Exposure to contaminated food/drinking water
- Environmental exposure

Case Classification

A. Possible case: NA
B. Probable case: Any person meeting the clinical criteria with an epidemiological link
C. Confirmed case: Any person meeting the clinical and the laboratory criteria

Note: If the national surveillance system is not capturing clinical symptoms, all laboratory-confirmed individuals should be reported as confirmed cases.

Figure 2.17.1 European Union case definition for hepatitis A cases, European Union, 2018. Source: European Commission 2018 (170)

EU/EEA countries' practices are not completely aligned. Generally, they involve, once the hepatitis A notification reaches the local public health office, taking contact with the patient to administer a questionnaire that collects epidemiological information, including patient's demographics, clinical information, history of exposures and risk factors for infection and further transmission. Such information is reported at the subnational and national level. The timing of data analysis greatly varies in the different EU/EEA countries (174). The use of more detailed patient's questionnaires in specific settings (e.g., genito-urinary medicine clinics) is useful surveillance enhancement to improve the monitoring of risk factors in a specific population (175). Furthermore, some countries perform additional forms of enhanced surveillance by matching epidemiological information with typing data from the strains detected in patients' samples (in 2016, 17 EU/EEA countries out of the 27 providing information had this capacity) or by matching epidemiological and viral information from human surveillance with typing data from strains identified in contaminated food (26).

Syndromic surveillance for hepatitis A (or for acute hepatitis) is generally performed in settings with high burden and sub-optimal surveillance. Thus, it is not commonly performed in the EU/EEA (173).

2.18 Event-based surveillance in the EU/EEA

Event-based surveillance (EBS) is an essential tool for the prevention and control of hepatitis A as it facilitates early detection of outbreaks, increasing the speed of implementation and the success of control measures. EBS is particularly useful when surveillance information is not rapidly collected and analysed, as it is the case for the hepatitis A surveillance at the European level, where hepatitis A IBS is not timely (Study 5).

Until 2021, European EBS was performed through an access-restricted web-platform named Epidemic Intelligence Information System for Food- and Waterborne Diseases and Zoonoses (EPIS-FWD) (176); such platform was upgraded in 2021 and named European Surveillance Portal for Infectious Diseases (EpiPulse) (177). EPIS/EpiPulse are communication platforms which EU/EEA public health authorities and global stakeholders (WHO and major centres for diseases controls) access to exchange information on potential infectious disease threats. In the case of hepatitis A, through the exchange of epidemiological and sequencing information, EU/EEA stakeholders are able to link sporadic cases to slowly evolving multicountry outbreaks associated with the same strain. Such outbreaks are most often associated to the exposure of the same contaminated food item internationally distributed, or to transmission in specific risk groups (e.g., MSM, travellers or ethnic minorities) (6).

Whilst EBS is not designed to assess trends of disease, through an analysis of the frequency and the extent of the outbreaks reported, it can still provide useful information on epidemic trends and associated population groups.

2.19 Hepatitis A outbreak investigations

The investigation of hepatitis A outbreaks follows practices like the investigation of outbreaks caused by other pathogens implicating foodborne or person-to-person transmission via the faecal-oral route. Such practices scholastically suggest 10 steps, including the confirmation of the outbreak, active case finding, the collection of patient's information for the purpose of a descriptive analysis and of analytical studies to test the hypotheses on the cause of the outbreak, and the use of parallel investigations to stimulate or corroborate the findings of the epidemiological investigation. Such parallel investigations include, for instance, laboratory investigations to sequence and phylogenetically analyse strains from humans, food and the environment, or environmental investigations to identify contaminated food items, compare consumer's habits or detect contaminated sewage waters (178-181). The outbreak case definition is an essential part of the investigation. Such case definition, pragmatically balancing specificity and sensitivity, differs from standard surveillance case definitions to allow for the collection of patient's information to clarify the person, place and time dimension specific to the event under investigation (182).

Large hepatitis A epidemics can be seen as a chain of local events. Local investigations are often focused on geographically defined point source outbreaks. National and international genome sequencing analysis and, in some instances, food safety investigations may play an essential role in uncovering the contribution of local outbreaks to prolonged common-source or propagated cross-border outbreaks (26).

Outbreak investigations aim to i) identify the cause and the transmission mode of an outbreak, and ii) implement mitigation and control measures. In regard to the first point, in foodborne outbreaks it is essential to identify not only the implicated vehicle of infection (e.g., a specific food item, or a food-outlet dish), but also the source of infection (e.g., contaminated water used to irrigate the implicated fruit, or the infected food-handler contaminating the implicated food-outlet dish), so to interrupt

prolonged and sometimes relapsing sources of infection (183). Regarding the second point, control measures include a range of interventions from risk communication to active immunisation or exclusion from work. In foodborne outbreaks, these include the removal of contaminated food items, or the implementation of good practices to eliminate or inactivate the virus from possible vehicles of infection (e.g., personal hygiene, water treatment, surface disinfection, food heat-treatment) (113). In person-to-person outbreaks, if a vaccination campaign is feasible, at-risk susceptible individuals are immunised with the objective to control the outbreak by reducing the susceptible population below herd-immunity thresholds (184). In all outbreaks, testing, contact tracing and immunisation of contacts of cases, particularly in the household, in educational institutions, in sexual networks and in food-establishments, are performed to reduce the number of secondary cases and break transmission chains (83). Last, risk communication, health promotion and community engagement are essential to educate the public and decrease the risk of infection (118).

3 RESEARCH AIMS

The aim of this thesis was to study the evolving hepatitis A epidemiology and the determinants of HAV transmission in the EU/EEA in order to provide recommendations for the improvement of the tools to monitor HAV infection and of the prevention and control strategies to mitigate its impact on the European population.

To achieve this, we conducted five epidemiological studies, each of these with a specific aim.

3.1 Specific aims

Study 1: Estimate HAV seroprevalence and susceptibility in the EU/EEA population.

Study 2: Describe the largest foodborne outbreak reported in Europe and demonstrate the European vulnerability to similar large and cross-border events.

Study 3: Describe the largest outbreak disproportionally affecting European MSM reported in Europe, highlighting the drivers of such event and how to mitigate similar threats in the future.

Study 4: Evaluate the hepatitis A surveillance in the EU/EEA, while describing the overall epidemiology of hepatitis A and providing recommendations for improvement.

Study 5: Assess whether the severity of hepatitis A increased from 1995 to 2014 in selected EU/EEA countries, highlighting the population groups at increased risk of a severe outcome.

4 MATERIALS AND METHODS

4.1 Data sources

The five studies made use of data from the literature, from specific epidemiological events and from national and European health data systems.

4.1.1 Serological studies

Serological studies reporting age-specific seroprevalence of anti-HAV IgG are a common tool used to inform on age-stratified population immunity as they indirectly provide a measurement of HAV infection incidence in specific time periods of the past (5). **Study 1** included serological studies performed in EU/EEA countries, available in English or other European languages, reporting primary results on age-specific anti-HAV IgG seroprevalence in human samples obtained from 1975 to 2014 from the general population (≥1 year of age), military recruits and blood donors. Studies on specific populations at increased risk of infection (e.g., sewage workers) were excluded to avoid overestimating the prevalence of anti-HAV IgG in a country population. Serological studies were retrieved through a systematic literature search in the databases Cochrane Library, Embase, Google Scholar, Medline (through PubMed) and SCOPUS, which collect scholarly literature on different subject fields including health and life science. A combination of searches in different scholarly databases is considered effective in identifying all or the vast majority of the available studies (185). A small number of additional serological studies included in Study 1 were identified in national reports and unpublished studies indicated by members of the ECDC network and the ECDC HAV expert panel.

4.1.2 Hepatitis A outbreak investigation databases

Outbreak investigation databases are ad hoc databases generated during the investigation of a hepatitis A outbreak. Information is sourced from different data collection systems, including laboratory reporting and patient's interviews, which may comprise information from trawling questionnaires and questionnaires for specific analytical studies. Information analysed in **Study 2** and **Study 3** was collected by national and sub-national public health authorities for routine public health work and specific outbreak control operations. The information was used to describe outbreaks and rapidly draw policies to mitigate and bring those under control. Part of the information was then transferred from the EU/EEA countries participating to the multicountry outbreak investigation to ECDC in anonymised (i.e., de-identified) format. Information transferred to ECDC included the patient's age, sex, country of reporting, time of symptoms onset, travel history, food exposures, sexual orientation, sexual practices, use of dating apps, clinical severity, vaccination status, HIV status and molecular information of the infecting strain.

4.1.3 The European Surveillance System (TESSy)

TESSy is the EU/EEA repository of surveillance data on selected infectious diseases. It is managed and maintained by ECDC since 2007 (186). TESSy data are collected by ECDC through an annual data call in the month of May and are then validated, analysed and disseminated by ECDC as per Article 3 of its Funding Regulations (187). Data dissemination occurs through annual epidemiological reports and through the ECDC Atlas of Infectious Diseases (2). For the period in analysis in **Study 4**, most information was from "case-based data", except for three EU countries reporting "aggregate-based data" for part of or the entire study period. Information available in TESSy for hepatitis A include case's demographic, exposure, clinical and diagnostic data. Study 4 included information on 29 EU/EEA

countries reporting hepatitis A information to TESSy (all current EU/EEA countries except Liechtenstein).

4.1.4 Eurostat databases

Eurostat is the statistical data provider of the European Union. Eurostat holds data on key comparable indicators for EU/EEA countries, including population data. EU/EEA national statistical authorities collect data that are shared with Eurostat, which validates, analyses and makes data across the EU/EEA comparable. Data are available as extractable databases or tables (188). In **Study 3**, **4** and **5** we used EUROSTAT estimates on mid-year annual EU/EEA country populations to calculate hepatitis A outbreak attack rates (Study 3) and notification rates (Study 4 and Study 5) expressed as number of cases per 100 000 population. In study 5 we also used information from the database "Certain infectious and parasitic diseases" to look at the length of hospitalisations due to infectious and parasitic diseases in different EU/EEA countries for the period from 2004 to 2014.

4.1.5 National hepatitis A surveillance data

Surveillance data for hepatitis A were extracted from Italian, Dutch, Norwegian, Spanish and Swedish national databases for the available periods and were made available by national public health authorities (Figure 4.7.1.). National hepatitis A surveillance data included case's time of reporting, sex and age-group. Such data were not extracted from TESSy because of the lack of hepatitis A data prior to 2007.

4.1.6 Electronic hospital discharge forms

Hospital discharge forms are health records collecting patient's medically relevant events occurring during the patient's hospitalisation. The electronic format facilitates exportation and linking of hospital forms filled across time and in different health facilities; however, the linking of data for the same patient depends on national design and capacity. Electronic hospitalisation discharge forms used in **Study 5** included data on case's time of hospitalisation, sex, age-group, length and number of hospitalisations, clinical outcomes and comorbidities for the available periods (Figure 4.7.1.). We obtained the Swedish data used in this study by merging the Swedish Inpatient Register with the Longitudinal integrated database for health insurance and labour market studies (LISA) based on individual identity number (189, 190). We obtained the resulting database in a de-identified format and the key-code was not made available to us. The Swedish Inpatient Register has a national coverage of 99% and made used of ICD-10 codes during the study period (189). LISA contains a wealth of information on demographics, education, employment and economic indicators for Swedish residents older than 16 years of age (190).

Hospitalisation data were extracted from hospital discharge forms based on International Statistical Classification of Diseases and Related Health Problems – 9th revision (ICD-9) codes ("070.1", HepA without hepatic coma, and "070.0", HepA with hepatic coma) and International Statistical Classification of Diseases and Related Health Problems – 10th revision (ICD-10) codes ("B15.9", HepA without hepatic coma, and "B15.0", HepA with hepatic coma). The International Statistical Classification of Diseases and Related Health Problems, in its different revisions, is an international conceptual framework created by WHO for the systematic collection, analysis and comparison of morbidity and mortality data collected over time in different countries (191).

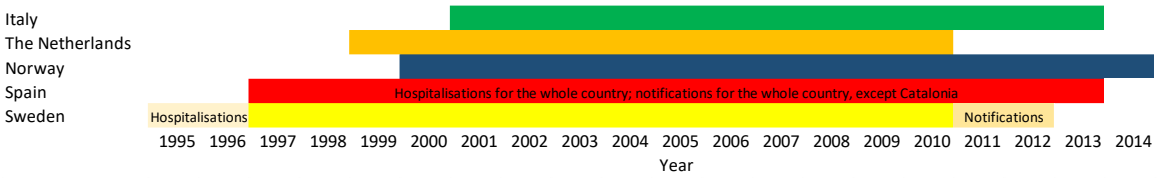


Figure 4.7.1. Available data for Study 5 on hepatitis A notification and hospitalisations by country and time period, and related geographical representativeness, in Italy, the Netherlands, Norway, Spain and Sweden, 1995-2014. Source: Severi E. 2022 (Study 5)

4.1.7 Ethical considerations

Information used in all studies was on human subjects. Study 1 was a meta-analysis of previously published literature. Study 2, 3, 4 and 5 used anonymised information collected for the EU/EEA population public health interest. Patient's information was collected at the national level and de-identified prior to the sharing with the study investigator. It is impossible to retrieve the identity or any additional information about the patients in the European studied because of the lack of a key-code linking the anonymised information with patient's identity. For study 2 and Study 3, national and subnational public health authorities collected informed consent from interviewed patients. Study 4 was an analysis of European surveillance data collected by ECDC. ECDC is an agency of the European Union with the mandate to strengthen Europe's defence against infectious diseases. Hepatitis A is one of the diseases for which EU surveillance is routinely performed through TESSy. As per Article 3 of the ECDC Funding Regulations and per Decision 1082/2013, ECDC collects, analyses and disseminates TESSy data (187, 192). Anonymised data are uploaded to TESSy by EU/EEA national authorities. Most information from TESSy is publicly available in the ECDC Surveillance Atlas of Infectious Diseases (2). Unpublished information appearing in Study 4 is presented at the supranational level and patient personal identifiable information cannot be extrapolated. Study 5 used information on patients' hospitalisation provided in an aggregated format by collaborating EU/EEA countries. Information on Swedish hospitalisations were extracted from the Swedish Inpatient Register and LISA after ethical review and related approval by the Stockholm Ethical Review Board.

4.2 Study designs

4.2.1 Meta-analyses

Meta-analyses are quantitative study designs statistically pooling together the results of studies identified through systematic literature reviews that independently reply to the same research question. Meta-analyses combine results of observational or interventional studies into a summary estimate supposed to represent the best evidence on the subject (193). In **Study 1**, a meta-analysis approach was used to combine results from studies estimating the anti-HAV IgG seroprevalence in the general population of EU/EEA countries over four decades. Information extracted from each study included age-specific seroprevalence estimates, time and country of sampling, study sample size and sampling approach. The meta-analysis provided age-specific summary estimates of seroprevalence for EU/EEA countries grouped in four susceptibility profiles. Susceptibility (or seronegativity) was defined as the inverse of seroprevalence. EU/EEA countries were allocated to one of the four susceptibility profiles based on the distribution of their seroprevalence estimates in the period 2000-2014 in adults aged 30 and 50 years.

4.2.2 Case-control studies

Case-control studies are retrospective observational studies comparing two study groups defined by the outcome of interest. Study participants who experienced the outcome are "cases", whereas those who did not are "controls". Study participants in the two groups are supposed to be drawn from the same defined population, so to have the same probability to experience the outcome. Once the two groups have been defined, the frequency of exposures of interest in cases and controls is compared to obtain measures of association, which are expressed as odds ratio (OR; i.e. the odds of exposure in cases versus the odds of exposure in controls). In matched case-control studies, controls can be matched to cases based on specific characteristics (e.g., demographics); matching four controls per case attains the maximum statistical power. Matched designs traditionally require a matched (conditional) analysis. Whilst it is often not recommended to adjust for the matching factors, this can be done through an un-matched (unconditional) analysis (193, 194). Well-designed case-control studies are efficient study designs that can be performed rapidly and inexpensively. **Study 2** reports the results of three matched case-control studies performed in Ireland, Italy and Norway studying the association of exposure to berries (i.e., the suspected vehicle of infection) with falling ill with the HAV outbreak strain of the 2013-2014 European foodborne outbreak.

4.2.3 Case-case studies

The case-case study design follows the same principles of the case-control design, with the difference that controls are drawn from cases (case-controls) falling ill with a disease caused by either a different subtype of the same pathogen (e.g., different strain, genotype, serotype) that caused the outbreak, or a different pathogen. It is essential that the population from which case-controls are drawn is the same or as similar as possible to the population of the outbreak cases. The period of exposure in the two groups should also overlap or be very closed in time. For an effective design, it is also paramount that the different subtypes or pathogens share the same risk factors for infection. Case-case studies offers the advantage of easily finding controls for whom patient's interviews are available, making the comparison between cases and controls extremely timely (195, 196). This opportunistic design is often used to investigate community outbreaks when the identification of controls is challenging, as can be the case when investigating hepatitis A outbreaks. In these investigations it is important to ensure that controls are susceptible to HAV infection. Ensuring the susceptibility of controls may only be attained through a serological test, as past infection could have been asymptomatic or vaccination certificates incomplete. Selecting controls amongst hepatitis A cases infected with HAV strains different either from the outbreak strain or, in multistrain outbreaks, from the outbreak strain of interest, ensures that control-cases were susceptible to HAV infection and, as hepatitis A has a rather long incubation period, that both cases and control-cases do not suffer differential recall bias. **Study 3** reports the results of an unmatched multicountry case-case study investigating whether cases infected with specific outbreak strains had different history of exposures during the large 2016-2017 cross-border hepatitis A outbreak disproportionally affecting MSM. The exposures of interest tested in the study were the number of sexual partners, the history of travel abroad and the use of dating apps. All models were also adjusted for age and vaccination status.

4.2.4 Cohort studies

Cohort studies are observational studies designed around study participants from a defined population who share a common exposure and is followed up to measure the study outcome. The design can be either retrospective or prospective, yet the study always follows the same direction in time so to measure

factors possibly associated with the study outcome when study participants have not yet experienced that. By comparing the frequency of exposures of interest in cases and non-cases study investigators obtain measures of association, which are expressed as incident rates or risk ratios (RR; i.e. the risk of exposure, or attack rate, in cases versus the risk of exposure in non-cases). The cohort design is efficient in investigating multiple study outcomes that can result from a single exposure, even when rare, as long as the study outcome is not a rare disease (193). **Study 4** reports the results of a retrospective cohort study where study participants were all notified or hospitalised hepatitis A patients in selected European countries and the outcomes of interest was severe hepatitis A.

4.3 Statistical methods

4.3.1 Descriptive epidemiology

Descriptive epidemiology is the first step in the analysis of epidemiological data. It entails the description of the study sample and of the outcome of interest, including its temporal and geographical distribution. Although using simple tools as counts and proportions (which can be used for the calculation of attack rates as well as incidence and prevalence measures), it can lead to the generation of statistical hypotheses to be tested in analytical studies. Descriptive epidemiology can also reinforce the evidence provided by other studies as well as monitor public health policies (193).

All studies made ample use of descriptive epidemiology; however, this was particularly important in outbreak investigations. Study 2 described the distribution of cases over the length of a foodborne outbreak, by reporting country, age, gender, travel history, clinical severity, food exposure and infecting strain; the descriptive analysis results were used to corroborate the findings from different national and cross-sectorial investigations (i.e. public health and food safety investigations) and to bring in additional evidence on the threats posed by foodborne outbreaks in Europe. Study 3 used descriptive epidemiology to confirm that an outbreak mostly affecting MSM was underway by estimating increases and male-to-female ratios in the early months of the outbreak compared with the same period in the previous four years. The study also characterised the distribution of cases associated with the multistrain outbreak by person, place and time and generated hypotheses on possible specific determinants of infection associated with the different HAV outbreak strains; such hypotheses were later tested in the analytical study. Study 4 and Study 5 presented data using time series analysis, looking at the frequency of hepatitis A notifications and hospitalisations over 10 and 20 years, respectively. In study 4, time series of the monthly frequency of hepatitis A notifications (or notification rates per 100 000 population), complemented with a linear trend and a 12-month moving average, were presented for all cases, by gender and by travel history, for the whole EU/EEA and for each group of country susceptibility profile. In Study 5, time series of the annual frequency of hepatitis A notification and hospitalisation rates per 100 000 population, complemented with a linear trend, were presented for all cases, for cases >40 years of age and for those with a clinically severe disease.

4.3.2 Logistic regression

Logistic regression models the association between one or more exposure variables (categorical or numerical) and a binary outcome. The measure of association is expressed through odds ratio (OR), computed as the odds of the outcome in the exposed group divided by the odds in the unexposed group. For categorical exposure variables with more than two categories, a reference category is generally set, and dummy variables are created to represent the odds in each category versus the reference group. For

continuous exposure variables instead, the parameter refers to the change in the outcome odds for each unit increase in the exposure variable (193).

In Study 1, we presented a curve of best fit to summarise age-specific seroprevalence estimates in four groups of EU/EEA countries over three time periods long 10 and 15 years. The curve of best fit allows for non-linear (S-shape) curves and is obtained through a generalised logistic regression model. This type of regression, an extension of logistic regression belonging to the family of multinomial logistic regression, uses maximum likelihood estimation to allow for outcomes not following a normal distribution to vary non-linearly through an arbitrary function of the outcome itself (193). In Study 1, the mid-points of age-specific seroprevalence estimates in each susceptibility group and period were fit as independent variables to model the different points of the curve of best fit (i.e. the outcome).

In Study 2, we reported on the use of conditional logistic regression to model the odds of exposure to suspected vehicles of infection (i.e., fresh or frozen soft fruits) in cases compared to the odds of exposure in matched controls. Associations were presented with ORs and the corresponding 95% confidence intervals (95% CI). Additional variables were fit into the models to adjust for the effect of confounders. Conditional regression is a branch of logistic regression where the exposure effect is estimated using the exposure distribution in strata of controls linked to specific cases (193).

In Study 3, we made use of unconditional logistic regression to model the odds of exposure to sexual practices, travel and vaccination history comparing cases infected with a specific outbreak strain (binary outcome yes/no) with cases infected with the other two outbreak strains. Overall, four models were developed: three models compared exposures in cases infected with a specific outbreak strain with cases infected with one of the other two outbreak strain; a fourth model compared cases infected with the strain most frequently reported in southern Europe with cases infected with the other two outbreak strains most frequently reported in central and northern Europe. Cases' age was also accounted for in the models. Associations were summarised with ORs and corresponding 95% CIs.

4.3.3 Negative binomial regression

Compared to logistic regression, negative binomial regression allows exposure variables and outcomes to be expressed as counts or rates and it is preferred to Poisson regression in case of overdispersion. The measure of association is expressed through an incidence rate ratio (IRR), computed as the rate in the exposed group divided by the rate in the unexposed group. As in logistic regression, for categorical exposure variables with more than two categories, a reference category is generally set and dummy variables are created to represent the odds in the each category versus the reference group, whereas for continuous exposure variables the parameter refers to the change in the outcome odds for each unit increase in the exposure variable (193).

In Study 5, we used negative binomial regression to model over the study period i) the annual rates of change in hepatitis A notification and hospitalisation rates; ii) annual rates of change in the age-group of notified and hospitalised hepatitis A patients; and iii) annual rates of change in patients with clinical severe hepatitis A. We also used negative binomial regression to assess risk factors for clinical severe hepatitis A in hospitalised patients. The outcome variable was the proportion of clinical severe disease in hospitalised patients (included as offset), and the explanatory variables were the patient's age group and the year of hospitalisation. The model was built testing the difference between coefficients before and after including an exposure variable. Each exposure variable was also tested with an interaction term and no effect modifier was identified.

5 RESULTS

5.1 Assessing HAV endemicity and susceptibility

Of the 4 276 unique records initially identified in the literature, we included 228 publications, entailing 279 unique studies (some publications included seroprevalence data from more than one study), from which we extracted 1 315 age-specific seroprevalence data points for which a population group, a country and a specific time of sampling could be defined. Such studies provided information for all EU/EEA countries, except Hungary, Latvia, and Lichtenstein.

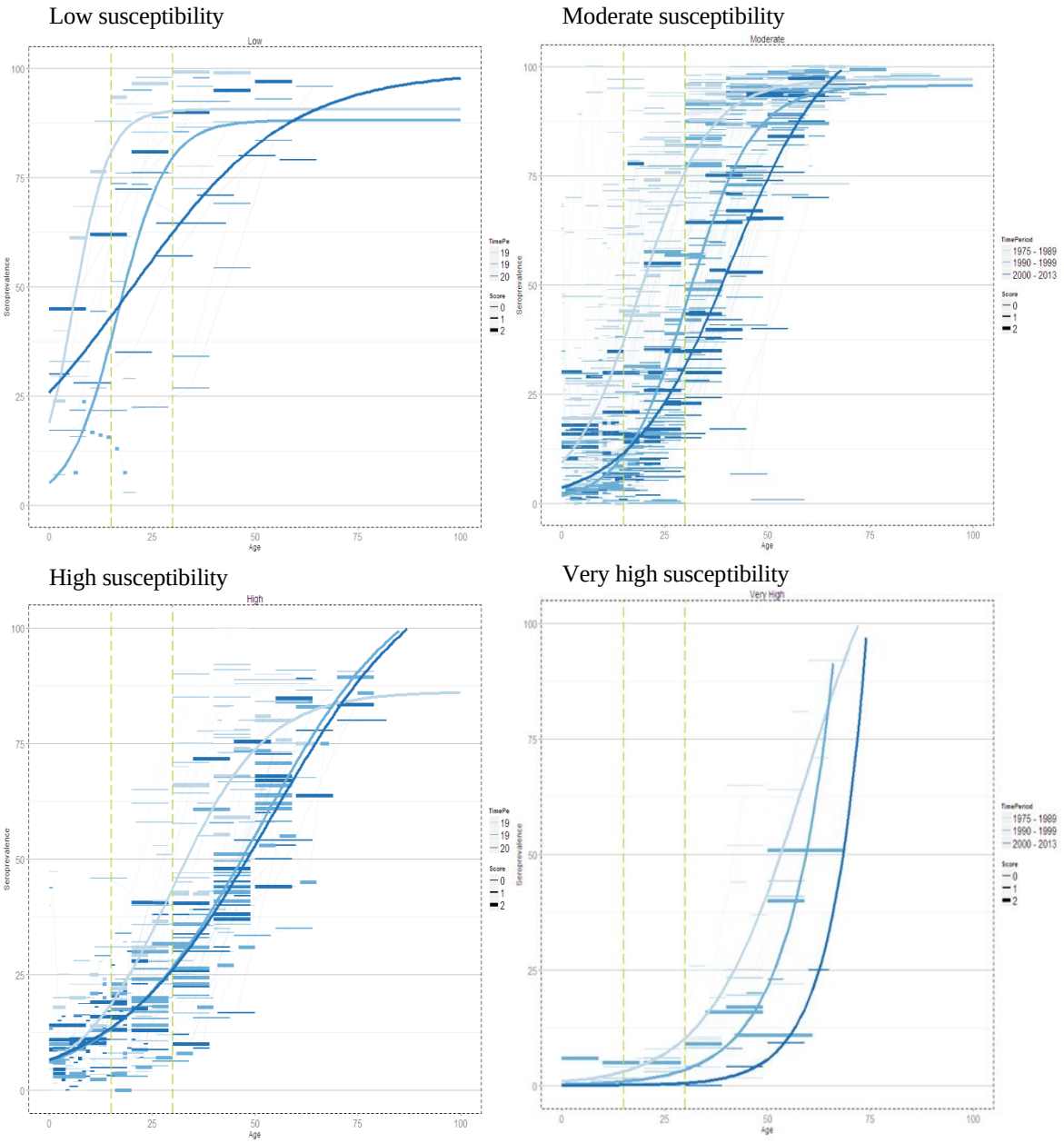


Figure 5.1.1. Age-specific HAV seroprevalence in the EU/EEA by susceptibility profile (low, moderate, high and very high) and time period (1975-1989, 1990-1999, 2000-2014), including a synthetic representation of the seroprevalence profiles (curve of best fit). Source: Carrillo-Santisteve P. 2017 (Study 1)

Age-specific seroprevalence data showed a decreasing trend in all countries over the study period. Such a shift was made visible in the shape of the curve of best fit, which had three different shapes in the different susceptibility profiles and over different periods (Figure 5.1.1):

- C-shape: seroprevalence levels of 75% or higher are reached in young adults before the age of 30 years, as it can be observed in countries with a low susceptibility profile from 1975 to 1999 and in those with a moderate susceptibility profile from 1975 to 1989.
- S-shape: seroprevalence levels of 75% or higher are reached in adults after the age of 30 years, as it can be observed in countries with a low susceptibility profile in the most recent study period, in those with a moderate susceptibility profile after 1989 and in those with a high susceptibility profile in all study periods.
- J-shape: seroprevalence levels of 50% are hardly reached at any age, as it can be observed in countries with a very high susceptibility profile in all study periods.

Countries' seroprevalence profiles showed a major shift from 1975-89, when only five countries (of 23 with available information) could be classified as having very low endemicity, to 2000-14, when 24 countries (of 28 with available information) were classified as having very low endemicity (Figure 5.1.2).

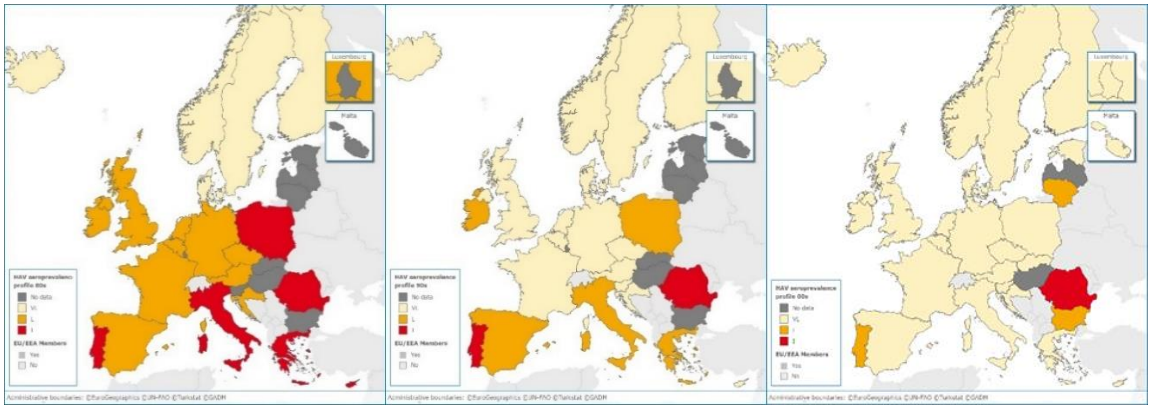


Figure 5.1.2. Geographical distribution of the HAV seroprevalence profiles in the EU/EEA in three periods, 1975-2014 (panel 1 includes studies with sampling year from 1975-1989, panel 2 studies from 1990-1999 and panel 3 from 2000-2014). Source: Carrillo-Santisteve P. 2017 (Study 1). VL=very low, L=low, I=intermediate. EU and EEA membership is shown for countries with no data.

For the last period (2000-14), the susceptibility profiles provided a more granular analysis on the proportion of adult susceptible population, resulting in four groups of countries with a high degree of geographical coherence (Figure 5.1.3).

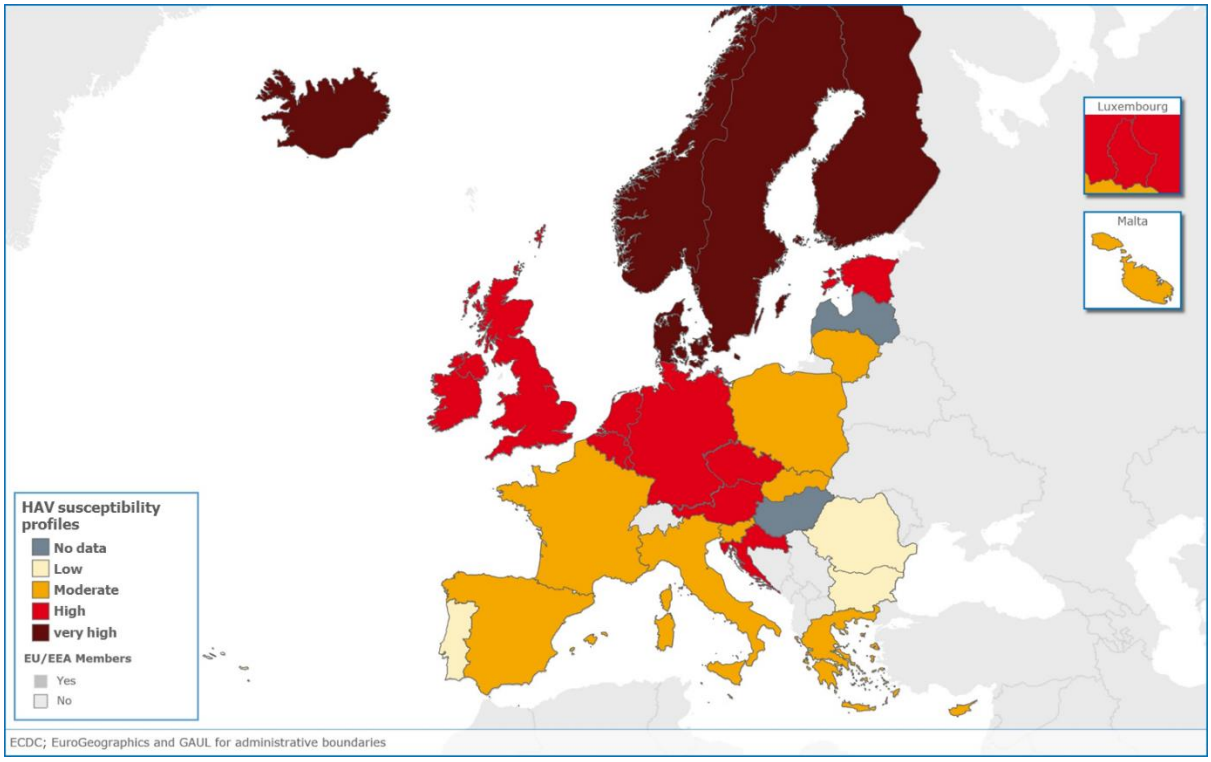


Figure 5.1.3. Geographical distribution of the HAV susceptibility profiles, EU/EEA, 2000-2014. Source: Carrillo-Santisteve P. 2017 (Study 1)

5.2 Describing a large and prolonged foodborne outbreak

The foodborne outbreak described in Study 2 was a prolonged common source foodborne outbreak associated with 361 confirmed and 1228 probable cases reported from 1 January 2013 to 31 August 2014 in 13 EU/EEA countries. Outbreak cases were defined based on the time of their onset of symptoms, their exposure history, the laboratory diagnosis and the genetic characterisation of the infecting strain.

During the outbreak investigations, only on a subset of EU/EEA countries performed genomic characterisation (i.e. partial genome sequencing) of the outbreak strain, which was at the basis of the definition for confirmed-case (Figure 5.2.1). Human samples with the same or very similar HAV sequence had strong evidence of a link to the source of the outbreak. The same HAV sequence was also identified in non-human HAV samples from food items (mixed berries and berry-products) identified in different EU/EEA countries.

Italy was the first and most affected country by the outbreak, reporting 90% of all cases. The outbreak peaked in Italy in April 2013, while the frequency of autochthonous cases peaked in July 2013 in Ireland, in October 2013 in the Netherlands, in February 2014 in Norway and in May 2014 in Finland, building up evidence on the serial distribution of contaminated products over almost two years and in several EU/EEA countries (Figure 5.2.2).

According to the European outbreak hepatitis A virus (HAV) infection case definition, a **confirmed case** is defined as:

An EU/EEA resident with laboratory-confirmed HAV genotype IA
and
date of symptom onset (or date of testing if onset date not available) on or after 1 January 2013
and
at least one of the following conditions:

- identical sequence (i.e. 100%) to the 2013 HAV genotype IA outbreak strain (GenBank accession number KF182323) based on a fragment of 460 nucleotides (nt) at the region of VP1-2a
- 99.8% similarity to this sequence (i.e. one nt difference in 460 nt) from 2,915 to 3,374 on NC_001489.
- identical sequence (i.e. 100.0%) on a shorter fragment of at least 174 nt at the region of VP1-2a from 2,967 to 3,191 on NC_001489.

According to the European epidemic HAV infection case definition, a probable (suspect/possible) case is defined as:
An EU/EEA resident with laboratory-confirmed HAV infection
and
date of symptom onset (or date of testing if onset date unavailable) on or after 1 January 2013
and
fulfilling, within 15–50 days before symptom onset, at least one of the following epidemiological criteria:

- having been in a country experiencing the outbreak during the indigenous outbreak period;
- person-to-person contact with a confirmed case (secondary case).

The following exclusion criteria for probable cases are applied:

1. HAV confirmed case who has a different sequence type to the 2013 HAV genotype IA outbreak strain;
2. existence of an epidemiological link to a person excluded for the reason given in criterion number 1;
3. history of travel outside EU/EEA/EFTA countries within 15–50 days before symptom onset.

Figure 5.2.1. European outbreak case definition, foodborne cross-border hepatitis A outbreak, European Union/European Economic Area 2013-2014. Source: Severi E. 2015 (Study 2)

About ¾ of the cases (n = 1,213) were in adults between 20 and 65 years of age (median age: 36 years; range: 1–92). Males were slightly more than females (ratio male to female: 1.15). There were 1,102 hospitalised patients, for a median duration of six days (range: 1–49; information on hospitalisation length available for 568 cases). Two deaths were reported as associated with this outbreak.

Case-control studies were performed in Ireland, Italy and Norway to identify the vehicle of infection. Chronologically, the Italian study first identified a significant association between outbreak cases and consumption of frozen berries (197). The Irish study confirmed such finding, pointing out that individuals that consumed frozen berries were 12 times more likely to fall sick compared to those who had consumed fresh berries (198). Finally, the Norwegian study reported on a significant association between outbreak cases and the consumption of a specific cake prepared using non-heat-treated frozen berries (199).

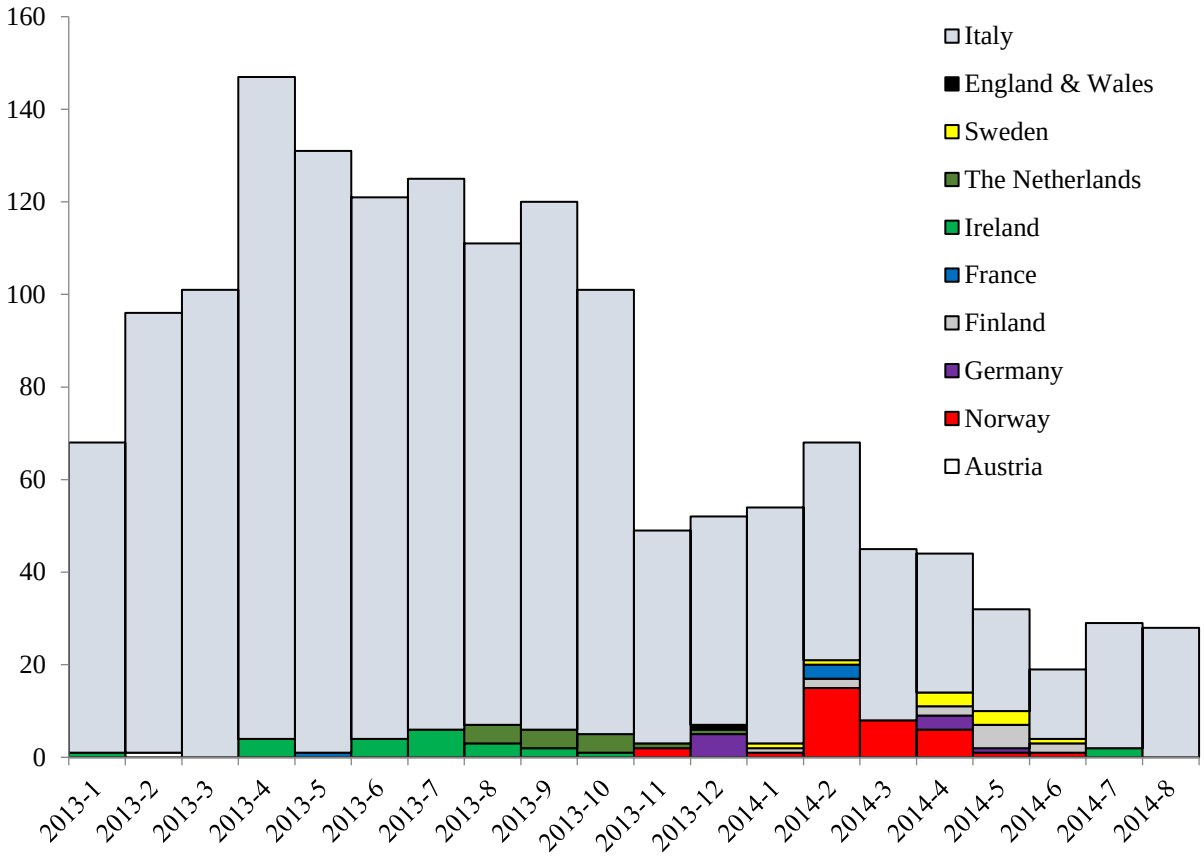


Figure 5.2.2. Hepatitis A cases by month of onset and probable country of infection, EU/EEA multistate foodborne outbreak, 1 January 2013 to 31 August 2014 (n=1587; 2 cases missing information on month of onset; date of testing was used when onset date was not available). Source: Severi E, 2015 (Study 2)

As a result of the outbreak investigation, national and European public health and food safety authorities implemented several response (e.g., risk communication, post-exposure prophylaxis and food recalls) and preparedness (e.g., promotion of a standard HAV sequencing protocol for human and food samples) measures.

5.3 INVESTIGATING A LARGE OUTBREAK DISPROPORTIONALLY AFFECTING MSM

From 1 June 2016 to 31 May 2017, Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Malta, the Netherlands, Norway, Portugal, Slovenia, Spain, Sweden and the UK reported 4 096 cases, of which 1 400 confirmed through genome sequencing, associated with a hepatitis A outbreak disproportionally affecting MSM.

The outbreak was confirmed through the analysis of notification rates from 1 June 2016 to 31 March 2017. Comparing with the same months in the previous four years, the study identified increases of at least two-fold in Austria, Greece, Italy, Malta, Portugal, Spain and the UK, confirming a multicountry outbreak was underway in several EU countries. The male to female ratio in cases aged 18 to 45 years was >3 in most of the reporting countries, confirming a predominance of male cases, and implying a disproportional MSM involvement in the outbreak.

The analysis of the outbreak cases showed that most cases were in adult male, particularly in Spain and Italy (52% and 19% of all cases, respectively). About half of the countries participating to the European investigation reported also probable and possible cases (Table 5.3.1).

	Confirmed (N=1400)	Probable (N=964)	Possible (N=1732)	Total (n=4096)
Gender				
Male	1283 (93%)[a]	964 (100%)[b]	1732 (100%)[b]	3941 (98%)
Age				
Median (IQR)	33 [28-43]	33 [27-40]	32 [26-38]	32 [27-40]
Age categories				
0-17 years	26 (2%)	4 (0%)	-	31 (1%)
18-45 years	1086 (79%)	864 (89%)	1732 (100%)[b]	3714 (90%)
46-65 years	244 (18%)	104 (11%)	-	348 (8%)
66+ years	22 (1%)	1 (0%)	-	23 (1%)
Reporting country				
Austria	26 (2%)	-	53 (3%)	79 (2%)
Belgium	42 (3%)	36 (4%)	40 (2%)	118 (3%)
Denmark	3 (0%)	4 (0%)	-	7 (0%)
Finland	8 (1%)	-	-	8 (0%)
France	294 (21%)	-	-	294 (7%)
Germany	98 (7%)	-	-	98 (2%)
Greece	4 (0%)	23 (2%)	15 (1%)	42 (1%)
Ireland	7 (1%)	5 (1%)		12 (0%)
Italy	175 (13%)	343 (36%)	279 (16%)	797 (19%)
Malta		7 (1%)	2 (0%)	9 (0%)
Netherlands	93 (7%)	10 (1%)	13 (1%)	116 (3%)
Norway	1 (0%)	-	-	1 (0%)
Portugal	109 (8%)	-	-	109 (3%)
Slovenia	4 (0%)	-	-	4 (0%)
Spain	262 (19%)	536 (56%)	1330(77%)	2128 (52%)
Sweden	11 (1%)	-	-	11 (0%)
United Kingdom	263 (19%)	-	-	263 (6%)

Table 5.3.1. Characteristics of hepatitis A cases by case classification, 1 June 2016 – 31 May 2017, participating EU/EEA countries (n = 4096). Source: Ndumbi P. 2018 (Study 3)

[a] Proportions are based on the total of available data for each variable

[b] As per outbreak case definition

By case definition, the 1 400 patients classified as confirmed cases were infected with one of the three outbreak strains. The geographical distribution of the three strains was characteristic: VRD_521_2016 was the most detected strain (56% of confirmed cases and predominant in southern Europe), followed by RIVM-HAV16–090 (35% of confirmed cases and predominant in central Europe and the UK) and V16–25801 (9% of confirmed cases and predominant in Germany) (Figure 5.3.1).

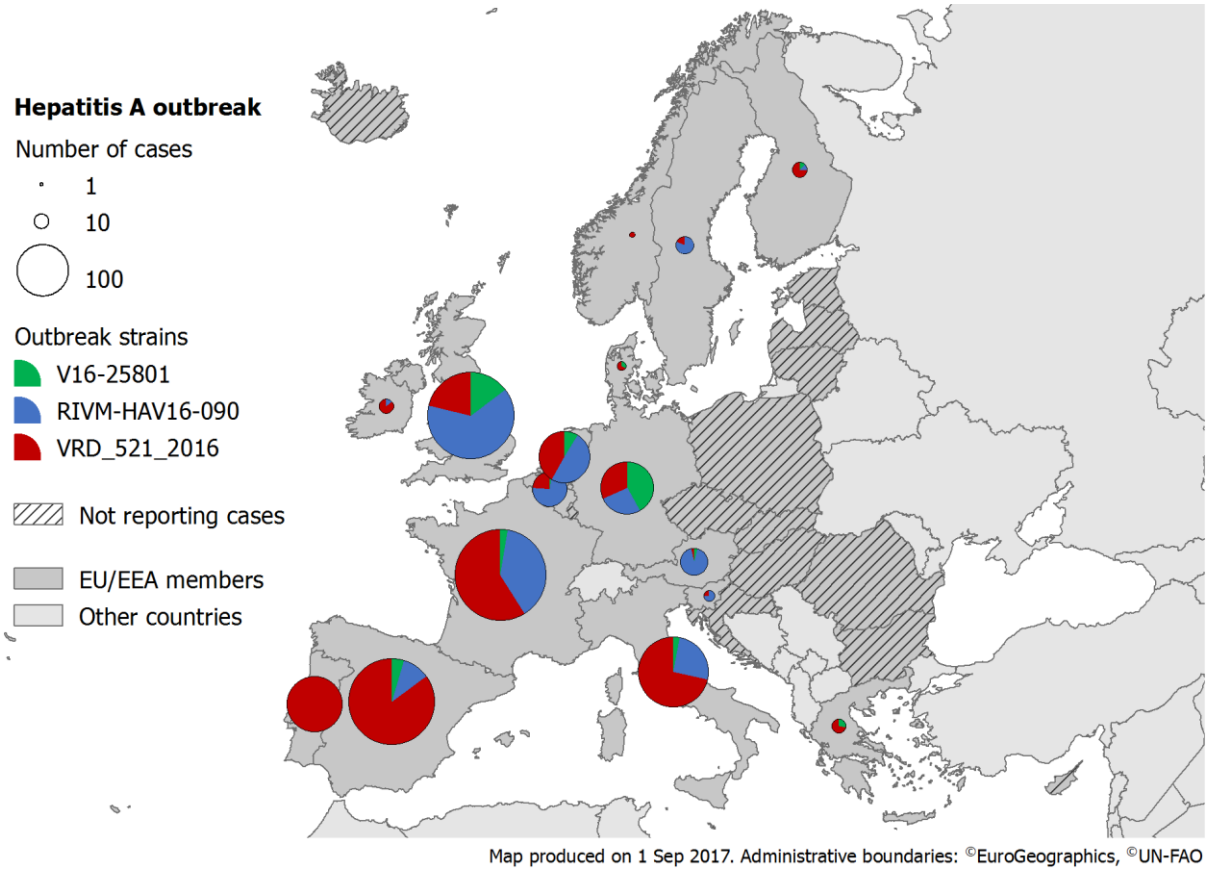


Figure 5.3.1. Distribution of confirmed cases by outbreak strain and EU/EEA reporting countries, 1 June 2016 – 31 May 2017 (n = 1 400). Source: Ndumbi P. 2018 (Study 3)

As for the rest of the cases, those classified as confirmed were mostly adult males self-reporting as MSM; about 40% were HIV positive and more than 50% had been hospitalised for at least one night. There were no significant differences neither in the abovementioned characteristics between confirmed cases infected with the three different strains, nor comparing the characteristics of the confirmed cases with those of the probable and possible cases in terms of demographics and travel and vaccination history (Table 5.3.1 and 5.3.2).

The four logistic regression models comparing individual outbreak strains with each other, and VRD_521_2016 with RIVM-HAV16 and 090/V16–25801 grouped together, did not detect any association between the different patients' sexual exposure and sexual practices and the specific strains, highlighting that no risk factors for infection with one specific outbreak strain were identified.

	RIVM (N=495)	V16 (N=119)	VRD (N=786)	Total (N=1400)	P-value[b]
Gender					0.623
Male	448(92%)[a]	112(94%)	723(93%)	1283(93%)	
Age					0.239
Median, IQR	34 [28-45]	34 [28-40]	33 [28-42]	33[28-43]	
Age categories					**0.001**
0-17 years	14(3%)	2(2%)	10(1%)	26(2%)	
18-45 years	358(73%)	96(83%)	632(82%)	1086(79%)	
46-65 years	101(21%)	18(16%)	125(16%)	244(18%)	
66+ years	15(3%)	0(0%)	7(1%)	22(2%)	
MSM					0.205
Yes	239(81%)	41(87%)	284(86%)	564(84%)	
HIV infection					0.551
Yes	28(42%)	1(20%)	31(45%)	60(43%)	
Hospitalisation					0.125
Yes	195(57%)	39(46%)	195(52%)	429(54%)	
Travel history					**0.001**
Yes	100(31%)	24(30%)	71(19%)	195(25%)	
Country of travel					0.335
Spain	31(31%)	9(38%)	29(41%)	69(35%)	
Germany	11(11%)	2(8%)	3(4%)	16(8%)	
Belgium	6(6%)	0(0%)	2(3%)	8(4%)	
Portugal	3(3%)	1(4%)	4(6%)	8(4%)	
Italy	1(1%)	1(4%)	5(7%)	7(4%)	
Other: EU	14(14%)	3(13%)	8(11%)	25(13%)	
Other: non-EU	22(22%)	3(13%)	9(13%)	34(17%)	
Multiple EU	8(8%)	5(21%)	7(10%)	20(10%)	
Multiple EU/non-EU	4(4%)	0(0%)	4(6%)	8(4%)	

Table 5.3.2. Characteristics of confirmed hepatitis A cases by strain in reporting EU/EEA countries from 1 June 2016 to 31 May 2017 (n=1400). Source: Ndumbi P. 2018 (Study 3)

[a] Proportions are based on the total of available data for each variable

[b] P-values are based on comparison between the three HAV strains using the chi-squared test for categorical variables and the Kruskal-Wallis test for continuous variables

5.4 EVALUATING THE EU/EEA HEPATITIS A SURVEILLANCE

From 2010 to 2019, 29 EU/EEA countries reported to TESSy 139 793 HepA confirmed cases. Eastern EU countries (particularly Bulgaria and Romania) reported the majority (>60%) of these cases. For the whole study period, the mean EU/EEA notification rate was 3.2 cases per 100 000 population, with a large peak in 2017 reaching 5.6 cases per 100 000 population. Country notification rates widely ranged over the study period: from 0 (in EU/EEA countries with a small population) to 75 cases per 100 000 population in Bulgaria in 2011 (Figure 5.4.1).

Overall, more male (57%) than female cases were reported; such proportion peaked in 2017 when 74% of the reported cases were in males. Time series analysis by gender shows an increasing trend in

notification rates in both groups, with the trend in males more accentuated. A peak is noticeable in 2014 in both genders, whilst the very large peak observed in 2017 is only in males (Table 5.4.1).

Country	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Mean 2010-2019
Austria	0.6	0.1	0.5	0.9	0.6	0.7	1.1	2.8	0.9	0.9	0.9
Belgium*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bulgaria	31.7	75.8	66.8	25.0	8.3	14.7	22.7	35.3	19.1	21.6	32.1
Croatia*	na	na	na	na	0.2	0.1	0.1	1.1	2.3	0.2	0.7
Cyprus	0.2	0.0	0.2	0.2	0.9	0.5	0.4	0.7	1.0	0.0	0.4
Czechia	8.2	2.5	2.7	3.3	6.4	6.9	8.8	7.3	2.0	2.3	5.0
Denmark	0.8	0.2	0.9	1.8	0.5	0.3	0.6	0.7	1.1	0.6	0.8
Estonia	0.5	11.5	4.8	0.5	0.9	0.5	0.5	3.4	1.1	1.5	2.5
Finland	0.3	0.3	0.1	0.8	0.5	0.8	0.1	0.5	0.5	0.3	0.4
France	1.9	1.7	1.7	1.4	1.4	1.1	1.0	5.1	2.3	2.0	2.0
Germany	0.9	1.0	1.0	1.0	0.8	1.0	0.9	1.5	1.3	1.0	1.0
Greece	0.5	0.4	0.7	1.4	0.8	0.6	1.9	2.6	1.0	0.3	1.0
Hungary	2.0	0.8	3.3	11.3	15.7	9.8	7.0	3.7	1.8	1.1	5.7
Iceland	0.6	0.3	1.3	0.0	0.0	0.0	0.0	1.5	0.3	0.6	0.5
Ireland	0.9	0.4	0.6	1.0	0.5	7.0	0.8	1.4	0.7	1.0	1.4
Italy	1.2	0.7	0.8	2.3	1.0	0.8	0.9	6.2	1.8	0.9	1.7
Latvia	13.8	2.4	0.5	0.6	1.0	0.3	0.5	3.8	3.5	1.9	2.8
Lithuania	0.3	0.6	3.8	2.2	0.6	0.2	0.6	1.3	0.5	0.3	1.0
Luxembourg	0.4	0.0	0.4	0.6	0.9	0.9	1.0	1.2	0.3	0.7	0.6
Malta	0.7	1.0	0.0	0.0	0.5	0.9	1.3	5.9	0.8	2.2	1.3
Netherlands	1.5	0.7	0.7	0.6	0.6	0.4	0.5	2.0	1.0	0.8	0.9
Norway	0.9	0.4	0.8	1.0	1.5	0.6	0.8	0.9	0.6	0.7	0.8
Poland	0.4	0.2	0.2	0.1	0.2	0.1	0.1	7.9	3.8	2.8	1.6
Portugal	0.1	0.1	0.1	0.1	0.2	0.3	0.5	5.4	0.8	0.4	0.8
Romania	17.2	12.8	17.9	20.8	33.3	26.0	16.1	12.6	23.2	17.3	19.7
Slovakia	26.9	7.4	2.3	3.8	13.6	16.3	25.0	12.4	3.2	1.8	11.3
Slovenia	0.4	0.5	0.5	1.1	0.5	0.2	0.6	1.7	0.8	0.6	0.7
Spain	1.6	1.0	1.2	1.3	1.3	1.2	2.8	9.7	4.9	2.1	2.7
Sweden	0.9	0.6	0.9	1.1	0.9	1.0	0.9	1.1	1.2	0.9	1.0
EU/EEA*	3.0	2.9	3.0	2.8	3.1	2.7	2.7	5.6	3.4	2.4	3.2

Lowest — Highest

Figure 5.4.1. Hepatitis A notification rates (cases per 100 000 population) by EU/EEA country and reporting year. Source: Severi E. 2022 (Study 4)

In each country row, annual notification rates are coloured ranging from light yellow to orange with darker colours indicating increasing notification rate values.

*Data for Belgium marked as "NA" (not applicable) because reporting from sentinel surveillance.

Data for Croatia marked as "na" (not available) before 2014 when entered the European Union.

Data for Belgium, for the whole study period, and for Croatia, before 2014, not included in the calculation of the EU/EEA notification rate.

Year 2017 is also the year with a peak in the median age of cases, shifting from 14-17 years in the period 2010-2016 to 31 years in 2017.

When stratifying countries by their HAV endemicity profile (see Study 1) two features appear. On one hand, the 2017 peak in males and in older cases, and the increasing trend in cases' frequency, is particularly evident in countries at moderate HAV susceptibility (79% males; 33 years of median age in 2017), whilst it is not visible in countries at low HAV susceptibility (57% males; 14 years of median age in the same year). On the other hand, a peak in both female and male cases, without a shift in the median age of infection, can be observed in 2013 and 2014 in countries at low and very high susceptibility (Figure 5.4.2).

Susceptibility region*	Age and sex	Reporting year										Median 2010-2019
		2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	
Low susceptibility	Median age	11	12	13	13	12	12	12	14	11	11	12
	% Males	54%	54%	54%	55%	55%	53%	56%	57%	54%	54%	54%
Moderate susceptibility	Median age	22.5	23	19	22	17	18	24	33	31	29	27
	% Males	59%	57%	54%	54%	54%	53%	63%	79%	62%	55%	64%
High susceptibility	Median age	23	30	29	33	33	25	30	33	38	40	31
	% Males	53%	54%	51%	51%	54%	53%	56%	65%	55%	53%	55%
Very high susceptibility	Median age	21	16	16.5	22	27	16	15	32.5	25.5	26	22
	% Males	54%	59%	48%	49%	56%	55%	54%	67%	54%	51%	54%
EU/EEA	Median age	16	17	16	17	14	14	17	31	24	22	20
	% Males	56%	55%	53%	54%	54%	53%	59%	74%	58%	54%	59%

Table 5.4.1. Hepatitis A cases' median age and proportion of males by hepatitis A virus susceptibility region and reporting year, EU/EEA from 2010 to 2019. Source: Severi E. 2022 (Study 4)

*Countries included in the analysis:

Low susceptibility: Hungary, Portugal, Romania.

Moderate susceptibility: Cyprus, France, Greece, Italy, Lithuania, Latvia, Malta, Poland, Slovenia, Slovakia, Spain.

High susceptibility: Austria, Croatia, Czechia, Estonia, Germany, Ireland, Luxembourg, the Netherlands.

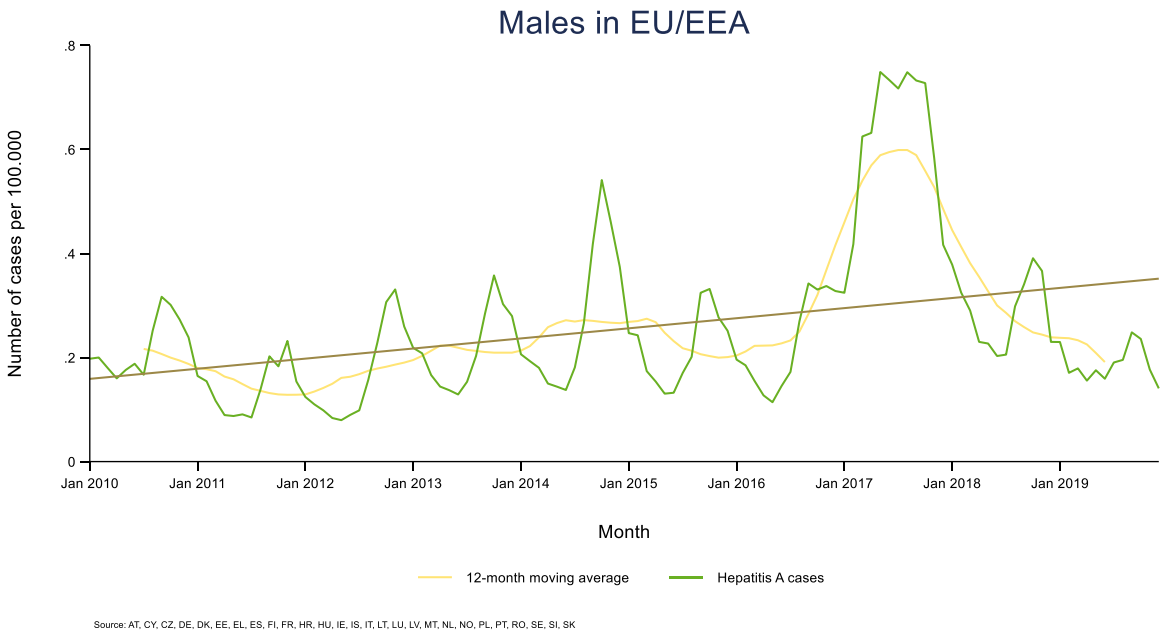
Very high susceptibility: Denmark, Finland, Iceland, Norway, Sweden.

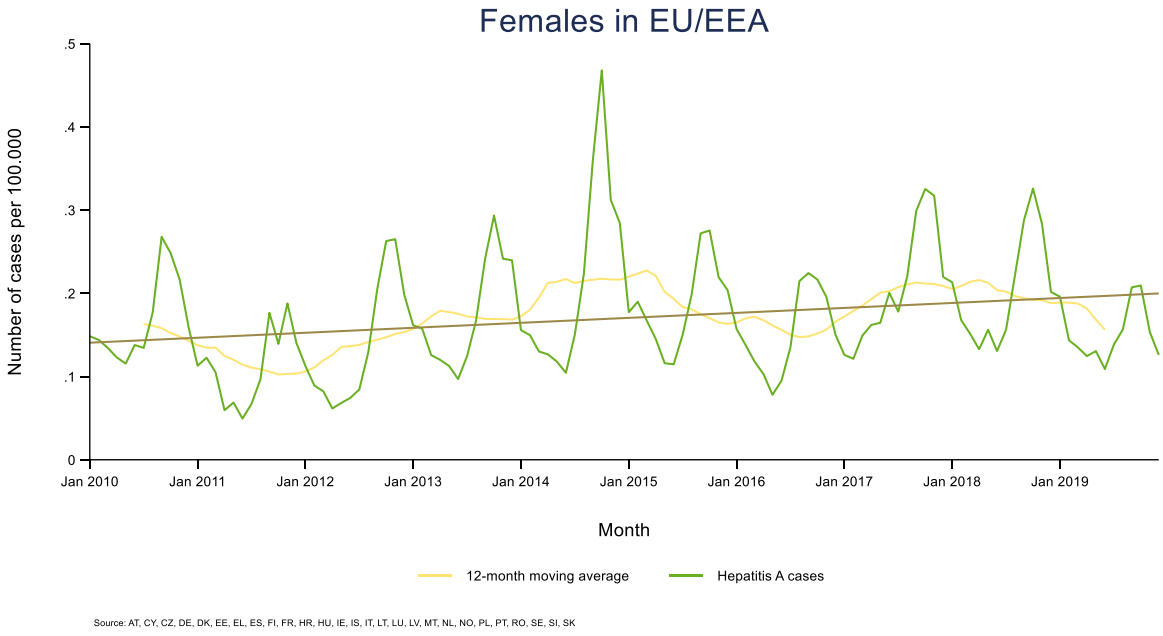
Travel to endemic countries is considered a major risk for HAV infection in the EU/EEA (127). About 15% of the cases with information on travel history were reported as travel-related. Hepatitis A suffers of a peculiar seasonality in Europe with travel-related cases peaking after the summer holidays and slightly anticipating the peak in non-travel related cases.

TESSy holds information on hospitalisations and deaths, yet with high variability in the completeness of these pieces of information amongst the EU/EEA countries. Hospitalisations peaked in 2014 and 2017, with the highest proportion of hospitalised patients amongst all reported cases in 2012, 2014, 2015 and 2019 (93%). Overall, 73 deaths were reported in EU/EEA, with Germany and Poland reporting more than half of these. The year with more deaths reported was 2017. In the countries reporting this information,

case-fatality ranged from 0.01% (in 2010) to 0.18% (in 2019). Deaths in patients >50 years of age were 54, of which almost two/thirds occurred between 2017 and 2019, with a case-fatality in patients older than 50 years of 0.6% in 2018 and 2019.

Information on HAV transmission route, travel history and case's outcome was poorly reported in TESSy for the majority of cases. The low completeness of such pieces of information limits their analysis and use.





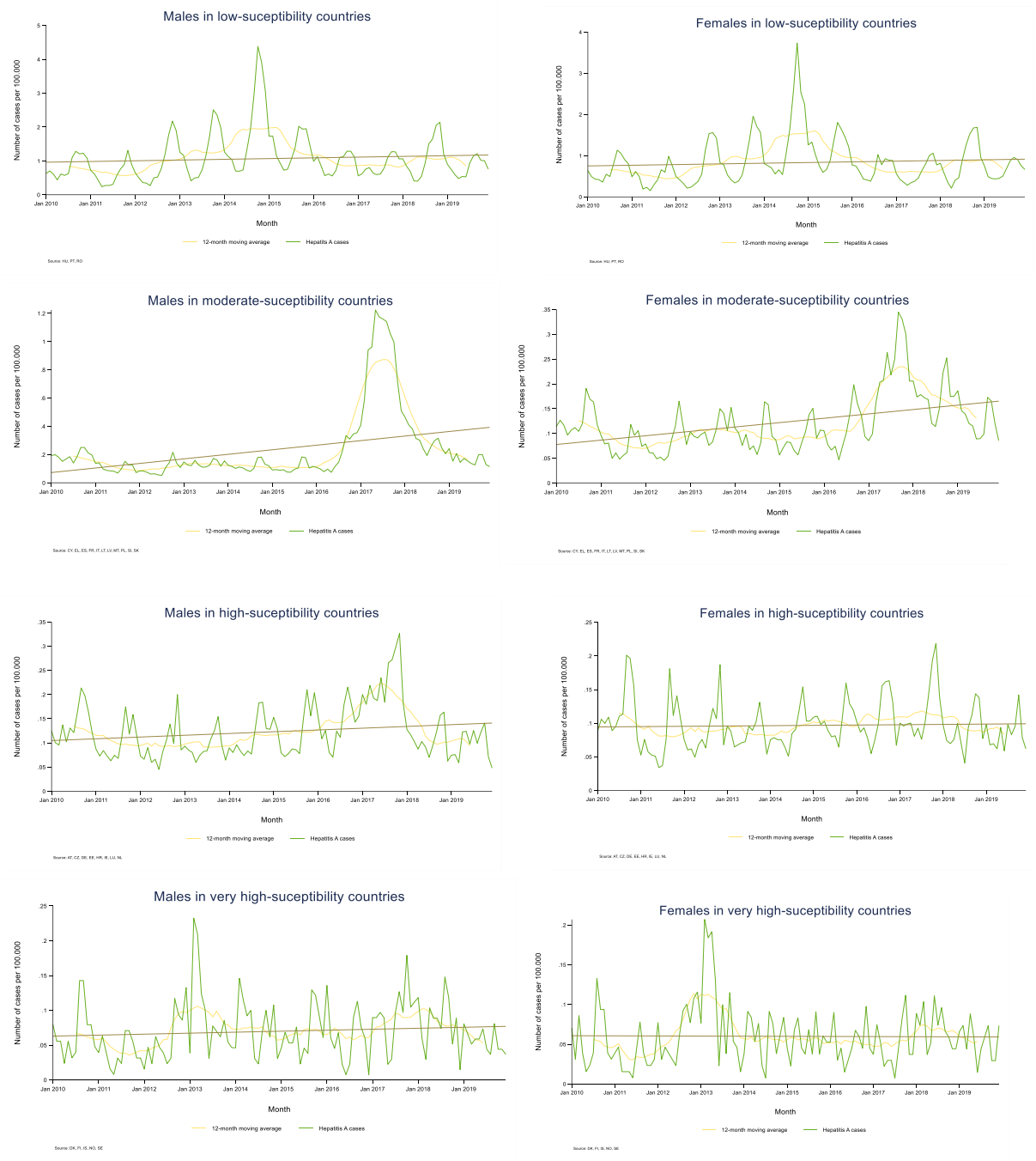


Figure 5.4.2. Distribution of hepatitis A notifications by 100 000 population, 12-month moving average and linear trend by reporting month and sex, from January 2010 to December 2019. Panel-A: female cases in all EU/EEA countries. Panel-B: male cases in all EU/EEA countries Panel-C: female cases in low hepatitis A virus susceptibility countries. Panel-D: male cases in low hepatitis A virus susceptibility countries. Panel-E: female cases in intermediate hepatitis A virus susceptibility countries. Panel-F: male cases in intermediate hepatitis A virus susceptibility countries. Panel-G: female cases in high hepatitis A virus susceptibility countries. Panel-G: male cases in high hepatitis A virus susceptibility countries. Panel-H: female cases in very high hepatitis A virus susceptibility countries. Panel-I: male cases in very high hepatitis A virus susceptibility countries. Source: Severi E. 2022 (Study 4)

5.5 Assessing trends in hepatitis A clinical severity

Study 5 included 36 734 notified and 36 849 hospitalised patients from Italy, the Netherlands, Norway, Spain and Sweden over available periods between 1995 and 2014 (Figure 5.5.1).

The study confirmed the hypothesised decrease in the notification and hospitalisation rates in Italy, the Netherlands, Norway and Sweden (with annual, mostly statistically significant decreases ranging from 4% in Dutch hospitalisations to 11% in Swedish notifications – Table 5.5.1).

Country-output	IRR	P	95% C.I.	
Italy-notifications	0.92	0.001	0.87	0.96
Italy-hospitalisations	0.90	<0.001	0.87	0.94
Netherlands-notifications	0.91	<0.001	0.86	0.95
Netherlands-hospitalisations	0.96	0.06	0.92	1.00
Norway-notifications	0.92	0.001	0.88	0.97
Norway-hospitalisations	0.94	0.001	0.90	0.97
Spain-notifications	1.03	0.23	0.98	1.09
Spain-hospitalisations	1.00	0.72	0.98	1.03
Sweden-notifications	0.89	<0.001	0.84	0.93
Sweden-hospitalisations	0.91	<0.001	0.88	0.95

Table 5.5.1. Incident risk ratio (IRR), p-value (p) and 95% confidence intervals (95% C.I.) of notification and hospitalisation rates by year, Italy, the Netherlands, Norway, Spain and Sweden, 1997-2014. Source: Severi E. 2022 (Study 5)

The study also confirmed the hypothesised increase in the age of notified patients in Italy, the Netherlands, Norway and Spain, and, in all countries but to a lesser extent, in the age of hospitalised patients (Table 5.5.2).

Country-output	IRR	P	95% C.I.	
Italy-notifications	1.08	<0.001	1.06	1.11
Italy-hospitalisations	1.02	<0.001	1.01	1.04
Netherlands-notifications	1.06	<0.001	1.04	1.08
Netherlands-hospitalisations	1.03	0.16	0.99	1.08
Norway-notifications	1.03	0.04	1.00	1.05
Norway-hospitalisations	1.01	0.60	0.98	1.03
Spain-notifications	1.11	<0.001	1.08	1.14
Spain-hospitalisations	1.07	<0.001	1.06	1.08
Sweden-notifications	0.99	0.52	0.97	1.02
Sweden-hospitalisations	1.04	<0.001	1.02	1.06

Table 5.5.2. Incident risk ratio (IRR), p-value (p) and 95% confidence intervals (95% C.I.) of the proportion of notified and hospitalised cases older than 40 years by year, Italy, the Netherlands, Norway, Spain and Sweden, 1995-2014. Source: Severi E. 2022 (Study 5)

Differently from what was initially hypothesised, the proportion of severe hospitalisations (fulfilling at least one of the following conditions during hospitalisation: death, liver transplant, hepatic coma, ≥3 courses of hospitalisations with hepatitis A, or hospitalisation length longer than seven days) did not increase over the study period. On the contrary, Italy, Norway and Spain observed a statistically significant decrease in the proportion of clinically severe hepatitis A patients (Table 5.5.3).

Country-output	IRR	P	95% C.I.	
Italy	0.98	<0.001	0.97	0.98
Netherlands	0.97	0.19	0.92	1.02
Norway	0.95	0.01	0.91	0.99
Spain	0.98	<0.001	0.97	0.99
Sweden	1.01	0.68	0.96	1.06

Table 5.5.3. Incident risk ratio (IRR), p-value (p) and 95% confidence intervals (95% C.I.) of the proportion of severe hospitalised patients by year, Italy, the Netherlands, Norway, Spain and Sweden, 1995-2014. Source: Severi E. 2022 (Study 5)

Last, with some degree of country variability, the study confirmed that patients with older age, with liver disease and with earlier year of hospitalisation were at increased risk of severe hospitalisation (Table 5.5.4).

Exposure		Crude IRR	P	95%CI	Adj IRR	P	95%CI
Italy							
Age-group	0-17	0.73	<0.001	0.69-0.78	**0.74**	**<0.001**	**0.70-0.78**
	18-39	Baseline					
	40-64	0.95	0.04	0.90-1.00	**0.95**	**0.02**	**0.91-0.99**
	65+	1.07	0.03	1.01-1.13	**1.06**	**0.04**	**1.00-1.12**
Co-morbidities	no co-morbidities	Baseline					
	liver disease	1.14	<0.001	1.07-1.22	1.04	0.16	0.98-1.10
	other co-morbidities	1.04	0.13	0.99-1.10	0.96	0.08	0.92-1.00
Year		0.98	<0.001	0.97-0.99	**0.98**	**<0.001**	**0.97-0.98**
The Netherlands							
Age-group	0-17	0.59	0.15	0.29-1.20	0.61	0.18	0.30-1.25
	18-39	Baseline					
	40-64	1.62	0.03	1.06-2.49	1.49	0.08	0.96-2.30
	65+	2.24	0.001	1.37-3.66	**1.88**	**0.01**	**1.14-3.11**
Co-morbidities	no co-morbidities	Baseline					
	liver disease	2.53	<0.001	1.66-3.87	**2.08**	**0.001**	**1.34-3.23**
	other co-morbidities	1.85	0.003	1.23-2.78	**1.64**	**0.02**	**1.08-2.50**
Year		0.97	0.19	0.92-1.02	**0.95**	**0.04**	**0.90-1.00**
Norway							
Age-group	0-17	0.64	0.34	0.30-1.34	0.65	0.26	0.31-1.37
	18-39	Baseline					
	40-64	1.52	0.06	0.99-2.35	**1.57**	**0.04**	**1.02-2.43**
	65+	2.72	<0.001	1.69-4.37	**2.69**	**<0.001**	**1.67-4.32**
Year		0.95	0.01	0.91-0.99	**0.95**	**0.02**	**0.92-0.99**
Spain							
Age-group	0-17	0.73	<0.001	0.62-0.85	**0.86**	**0.02**	**0.75-0.98**
	18-39	Baseline					
	40-64	1.44	<0.001	1.26-1.65	**1.39**	**<0.001**	**1.24-1.56**
	65+	2.34	<0.001	2.01-2.73	**2.10**	**<0.001**	**1.84-2.41**
Co-morbidities	no co-morbidities	Baseline					
	liver disease	3.15	<0.001	2.69-3.69	**2.58**	**<0.001**	**2.22-2.99**
	other co-morbidities	2.2	<0.001	1.91-2.54	**1.80**	**<0.001**	**1.58-2.05**
Year		0.98	0.001	0.97-0.99	**0.96**	**<0.001**	**0.95-0.97**
Sweden							
Age-group	0-17	1.12	0.67	0.66-1.92	1.68	0.06	0.97-2.90
	18-39	Baseline					
	40-64	1.82	<0.01	1.25-2.66	**1.71**	**0.01**	**1.17-2.48**
	65+	3.49	<0.001	2.34-5.19	**3.10**	**<0.001**	**2.08-4.62**
Co-morbidities	no co-morbidities	Baseline					
	liver disease	3.48	<0.001	2.28-5.31	**2.91**	**<0.001**	**1.88-4.52**
	other co-morbidities	3.11	<0.001	2.08-4.64	**2.52**	**<0.001**	**1.68-3.77**
Year		0.96	0.03	0.93-1.00	**0.96**	**0.01**	**0.93-0.99**

Table 5.5.4. Univariate (Crude) and multivariable (Adj) negative binomial regression models for the risk factor of severe hepatitis A hospitalisation, Italy, the Netherlands, Norway, Spain and Sweden, 1995-2014. Source: Severi E. 2022 (Study 5)

6 DISCUSSION

6.1 Main findings

6.1.1 HAV seroprevalence and susceptibility in Europe

Study 1 summarised and meta-analysed four decades of anti-HAV IgG age-specific seroprevalence estimates, highlighting patterns of historic HAV incidence over the 20th century in the EU/EEA and providing information on the time epidemiological transitions occurred in different EU/EEA areas. To our knowledge this is the first study providing such analysis for the whole EU/EEA.

The study also assessed susceptibility to HAV infection through the assessment of seronegativity in adults. This allowed allocating EU/EEA countries to one of four HAV susceptibility profiles. HAV susceptibility in adults is an epidemiological indicator offering a higher degree of discrimination than HAV endemicity. In fact, HAV endemicity, which is assessed as seroprevalence in children and young adults, provides information on virus circulation and incidence in the recent past and offers a uniform picture in most EU/EEA countries. Grouping European countries by HAV endemicity, Jacobsen et al. and WHO offer a uniform picture of the EU where most eastern EU countries are identified with low endemicity and the other countries with very low endemicity (5, 200, 201). However, HAV susceptibility allows for a more meaningful grouping of EU/EEA countries by capturing similar historic HAV infection incidence levels and providing current levels of HAV susceptibility in adults. Consequently, HAV susceptibility profiles allow to estimate the proportion of population at risk of symptomatic and clinically severe disease, and better define national and regional policy options.

The four HAV susceptibility profiles offered an effective tool to group countries in the analysis of surveillance data (see Study 4) and in placing in context the results of the other studies.

6.1.2 A large and prolonged foodborne outbreak

Study 2 described a foodborne outbreak associated with >1 500 cases in 13 EU/EEA countries over a period of almost two years. The investigation highlighted the large number of cases in clinically severe patients requiring hospitalisation and the vulnerability of the single European food market to large and prolonged HAV outbreaks. It also demonstrated the importance of the timely use of harmonised HAV sequencing protocols and of rapid sharing of sequencing results within the EU to prevent and rapidly respond to HAV infection outbreaks.

The foodborne origin of the outbreak was confirmed in several independent investigations performed at the national and subnational level (197-199). Our study brought together and reinforced scattered evidence, providing the full picture of a large and prolonged cross-sectorial investigation, which linked cases exposed in a very wide geographical area and infected by the same source of infection. The epidemiological investigation pointed at mixed berries as the vehicle of infection. Our findings were confirmed by an extensive trace-back exercise of contaminated products (202). The food safety investigations implicated, with strong evidence, two food products (black berries from Bulgaria and red currants from Poland) as possible vehicle of infection, but the food safety investigation could not identify which of the two was responsible for the primary contamination. In 2017 Bruni et al. published a study describing the HAV strains circulating in Bulgaria in 2012-2014, including the strain associated with a very large national hepatitis A outbreak occurring in Bulgaria since 2012 (203). The Bulgarian and the 2013-2014 European outbreak strain matched. The extent of the 2012 outbreak in Bulgaria and

its mode of transmission, mostly associated with person-to-person transmission, support the hypothesis that the vehicle of infection associated with the cross-border foodborne European outbreak was originating from Bulgaria (203). Soft fruit, even when produced within the EU, should be regarded as at-risk food items (116, 126, 204, 205).

6.1.3 A large outbreak disproportionally affecting MSM

Study 3 was a composite investigation. Using national surveillance data for the previous four years, it confirmed that a community hepatitis A outbreak mostly affecting male patients was taking place in the EU/EEA in late 2016 and early 2017. Through the analysis of genomic information, the investigation attributed the outbreak to three HAV strains with sub-genotype IA simultaneously circulating in the EU/EEA. The 1 400 patients infected with the outbreak strains were mostly unvaccinated MSM between 18 and 45 years of age engaging in risky sexual practices. Through a case-case study design, we analysed the characteristics of the patients infected with one of the three outbreak strains and did not find any difference in their risk factors for infection. Expanding the outbreak case definition to include cases for whom HAV sequencing had not been performed, the investigation described >4 000 cases probably or possibly participating to this outbreak in 17 northern, southern and western EU/EEA countries.

This investigation highlighted how intense and prolonged HAV transmission can be sustained by MSM networks in Europe and globally, with cases infected with the same strains identified mostly in men in at least three continents (118). An analysis of outbreaks reported in EU/EEA countries from 1997 to 2005 primarily affecting MSM assessed that international networks of MSM engaging in high-risk sexual practices can sustain HAV transmission for years (206).

The number of cases confirmed through sequencing in this outbreak was almost three times higher than in the 2013-2014 foodborne outbreak over a study period of almost half of the time (see Study 1). The large number of confirmed cases is partially due to the progresses and the success of the harmonisation of sequencing practices in EU/EEA countries. Multistrain HAV infection outbreaks have been reported in literature in association with contaminated food items or with travel to endemic countries (204, 207); however, detecting three HAV strains with considerable genomic differences associated with the same event is an interesting feature as large person-to-person multistrain HAV infection outbreaks are unusual. This outbreak most likely happened at a time when both proportion and mixing patterns of susceptible individuals engaging in high-risk sexual behaviours had grown larger than the threshold for population immunity. Zhang et al. estimated that an immunity level in MSM of 70% is necessary to avoid hepatitis A outbreak (208).

Not only the number of confirmed cases but also the number of probable and possible cases associated with this outbreak were very large. We identified more than the double of probable and possible cases in the 2016-2017 outbreak compared to the 2013-2014 foodborne outbreak. The results of the European Men-Who-Have-Sex-With-Men Internet Survey (EMIS) from 2010 indicated that MSM are exposed to risky sexual practices during international travels (209). Although WHO, ECDC and most EU/EEA countries recommended vaccination of MSM and of HIV positive patients, the extent of the outbreak showed the limited vaccine uptake in MSM at the start of this outbreak. To confirm this, the EMIS results from 2017 showed that only 43% of MS had been vaccinated for hepatitis A and 7% reported a history of hepatitis A (210). A shortage of hepatitis A vaccines mostly affecting European countries took place in 2016 and 2017. Communications between vaccine producers and national health authorities was possibly suboptimal. Adjustments in the prioritisation of vaccination groups and off-

label use of vaccines proved essential to compensate for this shortage (211); nonetheless, putting these adjustments in place took time and largely increased the outbreak extent, the time to control it and the direct and associated costs. A WHO/Europe report from 2020 and a review by Filia et al. published in 2022 highlighted that communications from vaccine producers on vaccine shortages are sub-optimal and need improvements at the global level (212, 213).

6.1.4 Hepatitis A surveillance in the EU/EEA

In Study 4 we provided a description of the epidemiology of hepatitis A in the EU/EEA and in the four HAV susceptibility groups of EU/EEA countries (see Study 1) from 2010 to 2019. Notification rates followed a gradient decreasing from east to south, and from west to north. Grouping countries based on their HAV susceptibility profile, which followed a similar pattern but were less sensitive to annual and national variations, provided a meaningful tool to describe different epidemiological situations within the EU/EEA.

Countries with a low HAV susceptibility profile (e.g Bulgaria and Romania) experienced the highest notification rates, driven by foodborne and person-to-person transmission. Similar drivers of transmission are documented by the authors of an analysis of the genetic diversity of HAV viruses sampled in Bulgaria in 2012-2014 (203, 214). The same analysis pointed out that a HAV strain circulating in Bulgaria since years matched the strain associated with the large foodborne outbreak affecting Italy and many other EU/EEA countries in 2013-2014 (203). Information on the causes of very large outbreaks like that occurring in Bulgaria in 2011-2012 or in Romania in 2014-2015 is scarce but appears characteristic of countries with an intermediate HAV endemicity, at least in those population groups having access to sub-optimal sanitation (5, 215).

Countries with a moderate and high HAV susceptibility profile (e.g southern and western EU countries) were the most affected by the 2017 outbreak. The analysis of TESSy data provided a picture of the full extent of the outbreak, which was associated with an excess of more than 10 000 cases and roughly doubled the number of cases expected for 2017. The same analysis showed how the outbreak continued in 2018. There is a wealth of literature from several EU countries showing how extensive and prolonged transmission was driven by international networks of MSM engaging in high-risk sexual behaviours and was amplified by spill-overs in the general community through foodborne or person-to-person transmission (103, 118, 208).

The lowest notification rates were reported in very high HAV susceptibility countries (i.e. northern EU/EEA countries), where infections were often associated to foodborne transmission or travel to endemic countries. Hepatitis A is a rare disease in these countries and infection clusters and outbreaks are rapidly detected and well documented (104, 205). The 2017 outbreak did not heavily affect this area, most likely because of high hepatitis A vaccination uptake in MSM and good risk communication and community engagement driven by public health authorities and civil society.

WHO recommends universal childhood vaccination in intermediate endemicity areas and vaccination of risk groups in very low and low endemicity areas (5). The Catalonian experience at the turn of this century showed how universal vaccination of toddlers, with catch up campaigns in older children, could rapidly decrease the HAV infection incidence in areas of sustained HAV transmission (216). Eastern EU countries like Bulgaria and Romania fit this situation and could rapidly benefit from such policy. In the rest of the EU/EEA, a large proportion of cases are known to be associated with travel to endemic countries or sexual behaviours facilitating faecal-oral transmission (127, 211). More aggressive

vaccination policies and awareness campaigns targeting these groups would significantly reduce the number of susceptible at-risk individuals, the frequency and the extent of large outbreaks and, consequently, the number of secondary cases and spill-overs in households, schools and through foodborne events (94, 103).

6.1.5 Trends in hepatitis A clinical severity

In study 5, we used digitalised health records from five selected countries in northern, southern and western EU/EEA to validate the hypotheses that the rate of hepatitis A notifications and hospitalisations decreased from 1995 to 2014, whereas the median age at infection increased. Similar findings were confirmed by a recently published study performed in a Spanish province between 1991 and 2017. Contrary to our third hypothesis, we did not identify an increase in the rate of clinically severe disease over the study period. On one hand, a similar picture was observed in the USA during an overlapping period (217). On the other hand, studies performed in Asia pacific countries, which underwent a more recent epidemiological transition, found a worsening trend in the clinical severity of hepatitis A (218, 219).

Our analysis of the risk factors for clinically severe disease identified patients with co-morbidities, and particularly chronic liver diseases, to be at higher risk of a clinically severe outcome. Such findings, consistent with previous knowledge, were also consistently identified by Sobotka et al. in an analysis of US hospitalisations from 2012 to 2014 (220). WHO recommends vaccination of patients with chronic liver disease (5). In 2021, Kronen et al. published a short report describing low rates (<50%) of hepatitis A immunity and opportunities for vaccination in a cohort of patients hospitalised with cirrhosis in Massachusetts (221). The situation in most EU/EEA is likely similar. Drivers of low vaccination offer should be addressed and corrected to improve access to hepatitis A vaccination in the patients most at-risk of severe disease.

6.2 Limitations

6.2.1 Selection Bias

Study 1 was based on a systematic literature review, which mostly included results from studies published in the literature. Small and local studies as well as studies offering conflicting or unexpected results may have been deprioritised and never seen publication. To reduce publication bias, in addition to seroprevalence studies published in scientific journals in all EU/EEA languages, we searched and included also studies published in public health agency's/ministry of health's websites and unpublished works known to members of the ECDC HAV expert panel. Studies included and meta-analysed in Study 1 did not offer a geographically homogeneous European sample over the four decades of the study period. Studies performed in Italy and Spain were very abundant, whereas studies from eastern and northern countries were scarce, particularly for more recent years (**Figure 6.2.1.1**). In countries with very few studies retrieved, local or not-optimally designed studies may have led to an inaccurate estimation of the age-specific seroprevalence within a country; however, we believe that the overall large number of studies have corrected for such inaccuracy within the group of countries with the same HAV susceptibility profile.

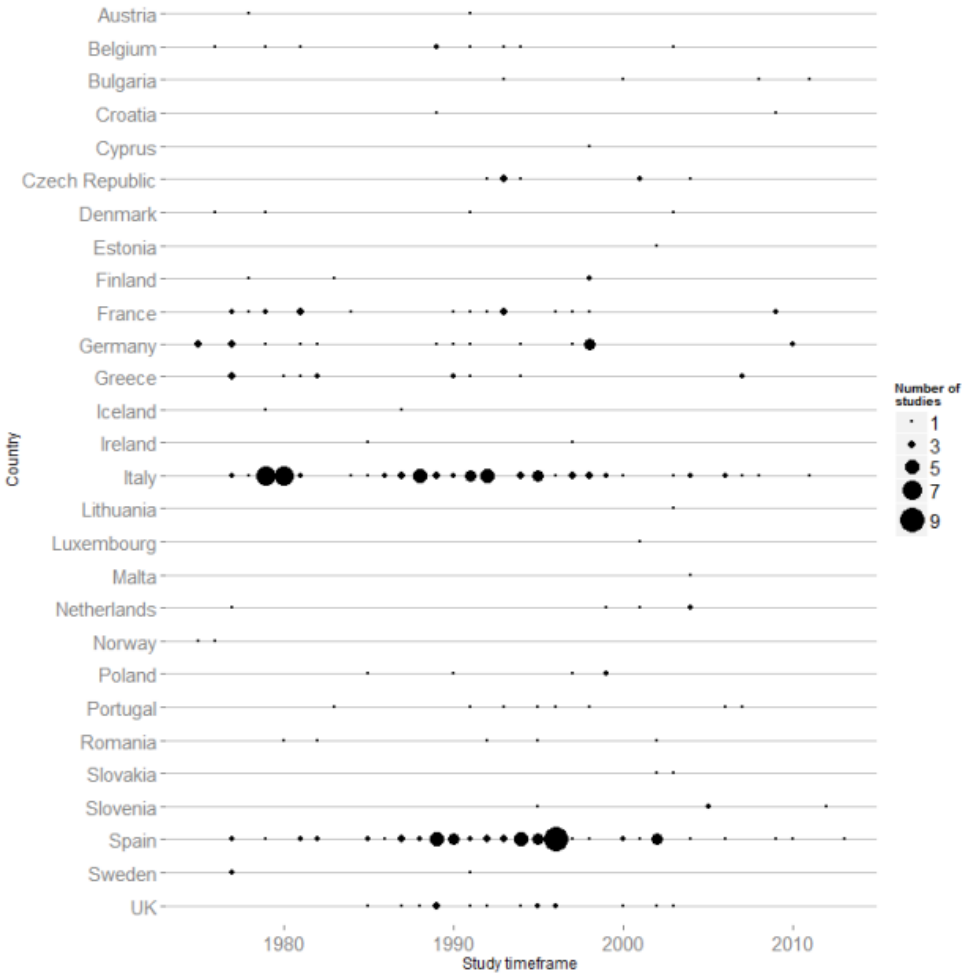


Figure 6.2.1.1. Distribution of seroprevalence studies included in Study 1 over the study period 1975 - 2014, by year of sampling. Source: Carrillo-Santisteve P. 2017 (Study 1)

The selection of the participants analysed in the two outbreak investigations (Study 2 and Study 3) was based on the respective outbreak case definitions. In both studies, the definition of confirmed cases was based on sequencing; however, practices related to the selection of samples to be sequenced are not aligned in the EU/EEA, with eastern EU countries performing sequencing at much lower rates, or not at all, than other EU/EEA countries. This resulted in an under-estimation of the true extent of the outbreaks and under-representation of cases from eastern EU. Furthermore, patients with more severe disease (e.g., requiring hospitalisation) were more likely to have samples undergoing sequencing, leading to an over-representation of older and more vulnerable patients and an under-estimation of younger individuals amongst the confirmed cases. Such bias affected also Study 4 and Study 5, where patients requiring hospitalisations were more likely to be reported to the national and European surveillance system.

Although different, both Study 2 and Study 3 definition of probable and possible cases may have led to the inclusion of cases actually not associated with the outbreaks under analysis, resulting in a possible over-estimation of the extent of the outbreak. Symmetrically, for many other cases the participation to the outbreak could have been missed owing to sub-optimal investigation and recall bias, resulting in a gross under-estimation of the true extent of the outbreaks.

Furthermore, in Study 2, the definition of probable case included information on past consumption of soft fruit. This is not a good practice. Otherwise, exposure to berries was not included in the case definition of the participating to the national case-control studies, therefore the odds of exposure to berries was not overestimated resulting in genuine measures of association.

Participants from the UK were over-represented in the case-case study reported in Study 3. Extrapolating those results for all other countries should be done with cautiousness.

Study 4 and Study 5 analysed surveillance data for hepatitis A. Hepatitis A surveillance suffers of under-ascertainment and under-reporting, resulting in an under-estimation of the true number of hepatitis A cases (222). Due to the large proportion of asymptomatic cases in children and mild cases in young adults, it is likely that TESSy grossly under-estimates the true number of HAV infections and, to a certain degree, also the number of hepatitis A cases. In Study 5, underreporting of hepatitis A notifications was a challenge particularly for Italy, where underreporting of infectious disease notifications to the national surveillance system is a known issue (223). However, such under-reporting is likely to be non-differential, as it can be observed by the consistency of the Italian results with those from the other countries included in the analysis.

In Study 4, because of the finding of Study 1, Portugal was grouped with low HAV susceptibility countries. Looking at the hepatitis A epidemiology in the last 10 years, Portugal could have fit better with countries at moderate or high HAV susceptibility. On the same note, Czechia and Slovakia have similarities with the countries at low HAV susceptibility (e.g., Bulgaria and Romania). We suggest caution when abstracting for these countries the results of the HAV susceptibility profile where they were placed.

In Study 5 we also used electronic hospital discharge forms. Italy, Norway and Sweden had a quasi-100% coverage of the national health facilities, whereas for the Netherlands such coverage was >80% and for Spain did not include Catalonia. We suppose that in all countries the information provided was representative of the whole population. However, a biased estimate of the association between exposure and outcome may have arisen if there were specific characteristics associated with the hepatitis A patients hospitalised in the health facilities not covered by the national data (e.g., higher socio-economical level, older age or lower frequency of clinically severe disease).

In the same study, results were from countries at very high, high and moderate HAV susceptibility and can be abstracted for most other countries with the same HAV susceptibility profile. The same cannot be done for those countries with low HAV susceptibility (i.e. Bulgaria and Romania), which underwent a more recent epidemiological transition.

6.2.2 Information bias

Hepatitis A vaccination is widely available since the late 90s of the last century, but diagnostic tools to discern between immunity induced by past infection or vaccination are not yet commonly in use. Owing to this, in Study 1 we were unable to discern the proportion of population immune to HAV because of past infection or immunisation, limiting our understanding of the historical pattern of HAV circulation in the EU/EEA.

The hepatitis A incubation period is long, lasting in average a full month. It may be challenging for individuals falling ill with hepatitis A to remember exposures occurred weeks prior to the onset of symptoms. Recall bias is common in hepatitis A patients (224), most likely affecting the outbreak

investigation reported in Study 2 and 3. However, such bias is non-differential and affected both cases and controls in the same way.

The foodborne and person-to-person transmission outbreaks described in Study 2 and Study 3 lasted for many months during which strong risk communication campaigns to inform the public of the risk of HAV transmission were implemented. Such knowledge may have biased both interviewers and patients in regard to reporting previous exposure to soft fruit in 2013-2014. Similarly, adult male patients may have been more likely to be diagnosed than adult females and children in 2017. In both cases, exposure to soft fruits and male adults were possibly over-represented in the finding of the two investigations.

Information on HIV status and history of sexually transmitted infections is not well captured during patient's interviews in the frame of an outbreak investigation, resulting in an under-estimation of the true proportion of HIV positive patients amongst the Study 3 participants. Similarly, information on past vaccination can be poorly recalled in absence of vaccination records, limiting the value of the analysis of the possible vaccination failures investigated in Study 3.

In Study 4, the sub-optimal completeness of information related to travel history, mode of transmission and death limits the use of surveillance data. Recording information on patient's death for hepatitis A may be challenging as patients may die relatively long after diagnosis and only some EU/EEA countries have a system in place to link deaths with previous notifications.

In Study 5, information on liver transplantation and multiple hospitalisations was not available for all countries. Because these pieces of information, amongst others, were used to define clinically severe cases, the countries missing this information (i.e., Norway and Spain) may have a lower number of severe cases and therefore slightly underestimate their rate. For all countries, information on biomarkers of liver injury was missing. Such information would have helped define clinically severe cases more consistently between the different countries and over the study period. Furthermore, we could not link hospitalisation and death registries, which could have led to a possible underestimation of the number of deaths and of severe outcomes identified in the study.

6.2.3 Confounding

The case-control studies reported in Study 2 were matched on sex (only in Ireland), age and area of residence. Matching on these characteristics was a way to control for their possible confounding effect. In Study 3, the multivariable analyses controlled for the possible confounding effect of age, vaccination, number of sexual partners, use of dating apps and travel history.

In Study 5 we controlled for age, year of hospitalisation and co-morbidity status. We also considered to control for the confounding effect of sex but excluding such factor from the model was leaving the other coefficients unaltered and we opted for a more parsimonious model not adjusting for sex. In Study 5, we assessed the possible effect of the year of hospitalisation (grouped in 5 year-periods) and of co-morbidities as effect modifiers in the association between age and clinically severe hepatitis A; after introducing an interaction term of the two variables (at different time), we performed a likelihood ratio test which suggested that neither of the two variables were interactions.

7 CONCLUSIONS

Systematically searching in the literature and pooling anti-HAV IgG seroprevalence studies with sampling from 1975 to 2014, Study 1 provided an indirect description of the historic HAV incidence over large part of the 20th century and indication on the time the epidemiological transition happened in most EU/EEA countries. Study 1 also delivered a discriminatory and innovative epidemiological indicator to group EU/EEA countries based on adult HAV susceptibility, paving the way to meaningful analyses of the epidemiology of hepatitis A in Europe.

In Study 2 we analysed the largest hepatitis A foodborne outbreak so far reported in Europe, highlighting the risk of clinically severe disease in adults and the vulnerabilities to contaminated food items (particularly frozen soft fruit) in the European common food market. This investigation was also a game-changer for the use of partial genome sequencing in hepatitis A outbreak investigations, leading to harmonised and reinforced practices in many EU/EEA countries.

In Study 3 we confirmed a multicountry outbreak disproportionally affecting MSM in Europe. We described the multistrain nature of this outbreak and identified no difference in the risk factors for infection with the different strains. We highlighted the very large extent that outbreaks associated with high-risk sexual practices can have, and the amplifying effect of vaccination shortages. The results of the investigation stressed the importance of ensuring high vaccine uptake in MSM since international networks of MSM proved to be able to sustain HAV transmission for very long.

In Study 4 we showed that hepatitis A is still a public health challenge in the EU/EEA. From 2010 to 2019, eastern EU countries had high notification rates and all countries were susceptible to large or very large foodborne or person-to-person transmission. This study placed the outbreaks described in Study 2 and Study 3 in context and provided the full picture of the 2017 outbreak extent. The study also highlighted TESSy limitations, mostly regarding completeness and data quality of travel history, route of transmission and clinical outcome.

Since surveillance data were not properly capturing information on clinical outcomes like length of hospitalisation, liver transplant and death, we analysed hepatitis A notifications and hospitalisations in five selected EU/EEA countries to assess whether the severity of hepatitis A presentation increased from 1995 to 2014. We confirmed that, during the study period, both notifications and hospitalisations decreased, that the age of notified and hospitalised cases increased and that the proportion of hospitalisations associated with clinically severe disease decreased. We also confirmed that older age and co-morbidities, particularly liver disease, are associated with increasing risk of clinically severe hepatitis A.

All studies depicted from different angles the benefits of harmonised EU studies and investigations.

8 POINTS OF PERSPECTIVE

Hepatitis A is still a public health challenge in the EU/EEA. In the last 10 years, very large national and cross-border outbreaks stood side by side to a baseline of cases driven by infections associated with travels to endemic countries and autochthonous person-to-person transmission. The EU/EEA countries experience different current and past epidemiological situations. On one hand, some strategies can apply to all countries; on another hand, tailored prevention and control measures are needed for specific contexts. These efforts should aim at both decreasing the number of baseline infections and avoiding outbreaks suddenly leading to large increases in cases.

Study 1 brought valuable information on HAV endemicity and susceptibility in the EU/EEA; however very few large seroprevalence studies have been conducted in the last 15 years. New studies should be performed by public health authorities and research groups using well-designed sampling strategies both at population-level and at the level of specific risk groups. Specifically, seroprevalence studies in MSM are needed to monitor immunity and implement vaccination campaigns when the immunity level drops <70%. Diagnostic tools to discern between immunity from natural infection and from vaccination are needed to make sense of new seroprevalence estimates to advance in policy planning. Lemon et al. called for an effort to finalise ongoing research to develop and commercialise sensitive immune assays already in 2017 (3).

In Study 2 and Study 3 we highlighted the essential role of HAV genome sequencing to characterise and link circulating HAV strains and detect occult outbreaks. Public health laboratories should perform timely sequencing on strains from a high proportion of cases, using the same European harmonised protocols both in the public health and in the food safety sector. Sequencing information should be rapidly shared internationally. Current European and global repositories of HAV sequences from human, food and the environment are either missing or are inefficiently used. In order to rapidly control outbreaks and foodborne transmission, new efforts should be placed to improve sequencing information sharing platforms as it is done for other foodborne pathogens by the ECDC and the European Food Safety Authority (225). Lemon et al. called for increased research on the possibilities offered by WGS to inform on the relatedness of different HAV genotypes and on global source identification during epidemiological investigations. Taking advantage of the large efforts put in place during the COVID-19 pandemic, the time appears mature to scale up HAV sequencing capacities and move to WGS to fully appreciate the possible mutations of the very well conserved HAV genome.

When an outbreak is detected, public health authorities should perform rapid and thorough investigations. Preparedness activities like dormient outbreak investigation protocols and capacity building can speed up outbreak control efforts. Multicountry investigations of cross-border events, where harmonised questionnaires and multicountry analytical studies are implemented in the participating countries, can bring substantial benefits to uncover the outbreak extent and better understand the implicated mode of transmission for all stakeholders involved in the investigation. Control measures implemented by public health authorities should include rapid testing and contact tracing to break transmission chains. National collaborations between public health and food safety authorities should be routinely carried on, and rapidly scaled up during foodborne outbreaks.

Study 4 demonstrated the need for public health authorities to routinely collect quality surveillance information in a case-based format. Such information should include the case's travel history (including the place of travel), sexual orientation, suspected mode of transmission, and disease outcomes like

hospitalisation and death. In a scenario of increasing susceptibility in older adults, to be able to monitor trends in the clinical presentation of hepatitis A, surveillance data should be fed also with information on liver transplants and cause of death collected in specific independent registries.

In Study 5 we studied trends in the severity of the hepatitis A clinical presentation in countries ranging from very high to moderate HAV endemicity. Similar studies should be repeated in countries at low HAV susceptibility (e.g., Bulgaria and Romania) that have undergone a more recent epidemiological transition and may be experiencing an increase in the number and the relative proportion of clinically severe HAV infection.

Overall, hepatitis A vaccination is essential to decrease HAV transmission and burden of disease. A range of strategies can be applied based on the 2012 WHO position paper and the recent European experiences (5). WHO recommends universal hepatitis A vaccination of toddlers in countries at intermediate endemicity. Countries like Bulgaria and Romania, based on up-to-date studies on seroprevalence and trends of hepatitis A severe disease, could likely benefit from this strategy. In countries at very low or low HAV endemicity, WHO, ECDC and EU/EEA countries recommend vaccination for groups at increased risk of infection and increased risk of severe disease. First, MSM should be a vaccination priority target; community engagement and risk communication should be scaled up to maximise opportunities for vaccination. Pre-emptive vaccination is assessed as the most cost-effective vaccination strategy and should be applied aiming at keeping immunity >70% in MSM (208). Reactive vaccination campaigns during outbreaks are also essential to rapidly increase immunity levels and speed outbreak control efforts. In such scenario, reinforced community engagement, health promotion and offer of vaccination in additional settings (e.g., saunas or MSM meeting venues) can bring substantial benefit. Second, efforts to vaccinate tourists and travellers (≥1 year of age) visiting friends and family in HAV endemic areas should be scaled up, as should the risk communication to increase vaccine acceptance and awareness in this group. Third, children of disadvantaged groups with sub-optimal access to safe water and sanitation should be vaccinated (along with improvements to the living conditions of such groups). Forth, patients with chronic liver disease should be offered vaccination to decrease the likelihood of clinically severe HAV co-infection. Hepatitis A vaccination shortages should be avoided or at least planned much in advance. During the 2017 shortage of hepatitis A vaccine, communication between vaccine producers and national health authorities proved to be sub-optimal. Improvements in the timeliness and channels to communicate on disruptions in the vaccine production are needed.

9 ACKNOWLEDGEMENTS

Over the course of my PhD I have received invaluable support from multiple angles. For this achievement I have to thank my principal PhD supervisor Johan Giesecke, my co-supervisors Pierluigi Lopalco and Lisen Arnheim Dahlström, and the MEB administrator Alessandra Nanni. I am grateful to my colleagues Julien Beauté, Edoardo Colzani, Emmanuel Robesyn, Gaetano Marrone, Theresa Enkirch, Marie Heloury, Lara Tavoschi, Paloma Carrillo Santisteve, Pontus Nauclér, Tarik Derrough, Therese Westrell, Johanna Takkinen, Agoritsa Baka and Josep Jansa, all my co-authors, and my mentors Koen De Schrijver, Claudio Badii and Yvan Hutin. I am very thankful to my parents, my siblings and my family in Italy and Brazil for their support from well before this journey started. Most of all, I want to thank my wife and my children for being on my side all the time.

10 REFERENCES

1. Cao G, Jing W, Liu J, Liu M. The global trends and regional differences in incidence and mortality of hepatitis A from 1990 to 2019 and implications for its prevention. Hepatol Int. 2021;15(5):1068-82.
2. European Centre for Disease Prevention and Control. Surveillance Atlas of Infectious Diseases Stockholm: ECDC; 2022 [Available from: https://atlas.ecdc.europa.eu/public/index.aspx.
3. 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. 2017;S0168-8278(17):32278.
4. World Health Organization. WHO immunological basis for immunization series: module 18: hepatitis A. 2019. In: Immunological basis for immunization series; module 18 [Internet]. Geneva: WHO. Available from: https://apliceps.who.int/iris/handle/10665/326501.
5. World Health Organization. WHO position paper on hepatitis A vaccines - June 2012. Geneva: WHO; 2012. Available from: https://www.who.int/publications/i/item/WER8728.
6. Gossner CM, Severi E, Danielsson N, Hutin Y, Coulombier D. Changing hepatitis A epidemiology in the European Union: new challenges and opportunities. Euro Surveill. 2015;20(16).
7. Shouval D. The History of Hepatitis A. Clin Liver Dis (Hoboken). 2020;16(Suppl 1):12-23.
8. Cockayne EA. Catarrhal jaundice, sporadic and epidemic, and its relation to acute yellow atrophy of the liver. QJM: An International Journal of Medicine. 1912;os6(1):1-29.
9. Epidemic hepatitis, or catarrhal jaundice. Journal of the American Medical Association. 1943;123(10):636-7.
10. Findlay GM, MacCallum FO, Murgatroyd F. Observations bearing on the aetiology of infective hepatitis (so-called epidemic catarrhal jaundice). Transactions of The Royal Society of Tropical Medicine and Hygiene. 1939;32(5):575-86.
11. Maccallum FO. Homologous Serum Hepatitis. Proc R Soc Med. 1946;39(10):655-7.
12. MacCallum F. 1971 International Symposium on Viral Hepatitis. Historical perspectives. Canadian Medical Association Journal. 1972;106(Spec Issue):423.
13. Gerlich WH. Medical Virology of Hepatitis B: how it began and where we are now. Virology Journal. 2013;10(1):239.
14. Krugman S, Giles JP, Hammond J. Infectious hepatitis. Evidence for two distinctive clinical, epidemiological, and immunological types of infection. Jama. 1967;200(5):365-73.
15. Feinstone SM, Kapikian AZ, Purceli RH. Hepatitis A: detection by immune electron microscopy of a viruslike antigen associated with acute illness. Science. 1973;182(4116):1026-8.
16. Dienstag JL, Feinstone SM, Kapikian AZ, Purcell RH. Faecal shedding of hepatitis-A antigen. Lancet. 1975;1(7910):765-7.
17. Ebert JW, Maynard JE, Bradley DW, Lorenz D, Krushak DH. Experimental infection of marmosets with hepatitis A virus. Primates Med. 1978;10:295-9.
18. Maynard JE, Krushak DH, Bradley DW, Berquist KR. Infectivity studies of hepatitis A and B in non-human primates. Dev Biol Stand. 1975;30:229-35.
19. Maynard JE, Lorenz D, Bradley DW, Feinstone SM, Krushak DH, Barker LF, et al. Review of infectivity studies in nonhuman primates with virus-like particles associated with MS-1 hepatitis. Am J Med Sci. 1975;270(1):81-5.

20. Provost PJ, Hilleman MR. Propagation of human hepatitis A virus in cell culture in vitro. Proc Soc Exp Biol Med. 1979;160(2):213-21.
21. 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(27):11223-8.
22. Provost P, Gerety R, Hilleman M, McAleer W, Scolnick E, Bishop R, et al. New findings in live, attenuated hepatitis A vaccine development. J Med Virol. 1986;20(2):165-75.
23. Fangcheng Z, Xuanyi W, Mingding C, Liming J, Jie W, Qi J, et al. Era of vaccination heralds a decline in incidence of hepatitis A in high-risk groups in China. Hepat Mon. 2012;12(2):100-5.
24. Cohen JI, Ticehurst JR, Purcell RH, Buckler-White A, Baroudy BM. Complete nucleotide sequence of wild-type hepatitis A virus: comparison with different strains of hepatitis A virus and other picornaviruses. J Virol. 1987;61(1):50-9.
25. Brown EA, Jansen RW, Lemon SM. Characterization of a simian hepatitis A virus (HAV): antigenic and genetic comparison with human HAV. J Virol. 1989;63(11):4932-7.
26. Enkirch T, Severi E, Vennema H, Thornton L, Dean J, Borg ML, et al. Improving preparedness to respond to cross-border hepatitis A outbreaks in the European Union/European Economic Area: towards comparable sequencing of hepatitis A virus. Euro Surveill. 2019;24(28).
27. Scholz E, Heinricy U, Flehmig B. Acid stability of hepatitis A virus. J Gen Virol. 1989;70 (Pt 9):2481-5.
28. Siegl G, Weitz M, Kronauer G. Stability of Hepatitis A Virus. Intervirology. 1984;22(4):218-26.
29. 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(3):159-63.
30. 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(7445):367-71.
31. 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(7532):85-8.
32. Feng Z, Hirai-Yuki A, McKnight KL, Lemon SM. Naked Viruses That Aren't Always Naked: Quasi-Enveloped Agents of Acute Hepatitis. Annual Review of Virology. 2014;1(1):539-60.
33. Lemon SM, Binn LN. Antigenic relatedness of two strains of hepatitis A virus determined by cross-neutralization. Infect Immun. 1983;42(1):418-20.
34. Aragonès L, Bosch A, Pintó RM. Hepatitis A virus mutant spectra under the selective pressure of monoclonal antibodies: codon usage constraints limit capsid variability. J Virol. 2008;82(4):1688-700.
35. International Committee on Taxonomy of Viruses. Positive-sense RNA Viruses - Picornaviridae - Genus: Hepatovirus: ICTV; 2022 [Available from: https://talk.ictvonline.org/ictv-reports/ictv_online_report/positive-sense-rna-viruses/w/picornaviridae/709/genus-hepatovirus.
36. Costa-Mattioli M, Ferré V, Casane D, Perez-Bercoff R, Coste-Burel M, Imbert-Marcille BM, et al. Evidence of recombination in natural populations of hepatitis A virus. Virology. 2003;311(1):51-9.
37. 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.

38. Robertson BH, Jansen RW, Khanna B, Totsuka A, Nainan OV, Siegl G, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. J Gen Virol. 1992;73 (Pt 6):1365-77.
39. Kroneman A, de Sousa R, Verhoef L, Koopmans MPG, Vennema H, On Behalf Of The HN. Usability of the international HAVNet hepatitis A virus database for geographical annotation, backtracing and outbreak detection. Euro Surveill. 2018;23(37).
40. Ramachandran S, Xia GL, Dimitrova Z, Lin Y, Montgomery M, Augustine R, et al. Changing Molecular Epidemiology of Hepatitis A Virus Infection, United States, 1996-2019. Emerg Infect Dis. 2021;27(6):1742-5.
41. Abe H, Ushijima Y, Bikangui R, Ondo GN, Zadeh VR, Pemba CM, et al. First evidence for continuous circulation of hepatitis A virus subgenotype IIA in Central Africa. J Viral Hepat. 2020;27(11):1234-42.
42. Endo K, Inoue J, Takahashi M, Mitsui T, Masuko K, Akahane Y, et al. Analysis of the full-length genome of a subgenotype IIIB hepatitis A virus isolate: primers for broadly reactive PCR and genotypic analysis. J Med Virol. 2007;79(1):8-17.
43. Endo K, Takahashi M, Masuko K, Inoue K, Akahane Y, Okamoto H. Full-length sequences of subgenotype IIIA and IIIB hepatitis A virus isolates: characterization of genotype III HAV genomes. Virus Res. 2007;126(1-2):116-27.
44. D'Andrea L, Pérez-Rodríguez FJ, de Castellarnau M, Manzanares S, Lite J, Guix S, et al. Hepatitis A virus genotype distribution during a decade of universal vaccination of preadolescents. Int J Mol Sci. 2015;16(4):6842-54.
45. Miyamura T, Ishii K, Kanda T, Tawada A, Sekimoto T, Wu S, et al. Possible widespread presence of hepatitis A virus subgenotype IIIA in Japan: Recent trend of hepatitis A causing acute liver failure. Hepatol Res. 2012;42(3):248-53.
46. 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.
47. Pintó RM, Pérez-Rodríguez FJ, Costafreda MI, Chavarria-Miró G, Guix S, Ribes E, et al. Pathogenicity and virulence of hepatitis A virus. Virulence. 2021;12(1):1174-85.
48. Koff RS. Hepatitis A. Lancet. 1998;351(9116):1643-9.
49. Ibáñez-Tomás E, Gasch-Gallén À. Sexual practices and the risk of Hepatitis A in men who have sex with men in Spain. J Nurs Manag. 2021;29(1):32-42.
50. Ndumbi P, Freidl GS, Williams CJ, Mårdh O, Varela C, Avellón A, et al. Hepatitis A outbreak disproportionately affecting men who have sex with men (MSM) in the European Union and European Economic Area, June 2016 to May 2017. Euro Surveill. 2018;23(33).
51. Purcell RH, Wong DC, Shapiro M. Relative infectivity of hepatitis A virus by the oral and intravenous routes in 2 species of nonhuman primates. J Infect Dis. 2002;185(11):1668-71.
52. Shouval D. Hepatitis A virus. In: Kaslow R.A., Stanberry L.R., Le Duc J.W., editors. Viral Infections of Humans - Epidemiology and Control. New York: Springer; 2014.
53. 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(3):260-8.
54. 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(8):6621-8.
55. 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(16):4282-96.

56. Das A, Barrientos R, Shiota T, Madigan V, Misumi I, McKnight KL, et al. Gangliosides are essential endosomal receptors for quasi-enveloped and naked hepatitis A virus. Nat Microbiol. 2020;5(9):1069-78.
57. Das A, Hirai-Yuki A, González-López 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(5).
58. Hirai-Yuki A, Hensley L, Whitmire JK, Lemon SM. Biliary Secretion of Quasi-Enveloped Human Hepatitis A Virus. mBio. 2016;7(6).
59. Lemon SM, Murphy PC, Shields PA, Ping LH, Feinstone SM, Cromeans T, et al. Antigenic and genetic variation in cytopathic hepatitis A virus variants arising during persistent infection: evidence for genetic recombination. J Virol. 1991;65(4):2056-65.
60. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. J Infect Dis. 2000;182(1):12-7.
61. Lanford RE, Walker CM, Lemon SM. The Chimpanzee Model of Viral Hepatitis: Advances in Understanding the Immune Response and Treatment of Viral Hepatitis. Ilar j. 2017;58(2):172-89.
62. Lemon SM, Binn LN, Marchwicki R, Murphy PC, Ping LH, Jansen RW, et al. In vivo replication and reversion to wild type of a neutralization-resistant antigenic variant of hepatitis A virus. J Infect Dis. 1990;161(1):7-13.
63. Glikson M, Galun E, Oren R, Tur-Kaspa R, Shouval D. Relapsing hepatitis A. Review of 14 cases and literature survey. Medicine (Baltimore). 1992;71(1):14-23.
64. 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(1):9-19.
65. Rezende G, Roque-Afonso AM, Samuel D, Gigou M, Nicand E, Ferre V, et al. Viral and clinical factors associated with the fulminant course of hepatitis A infection. Hepatology. 2003;38(3):613-8.
66. Schoch S, Wälti M, Schemmerer M, Alexander R, Keiner B, Kralicek C, et al. Hepatitis A Virus Incidence Rates and Biomarker Dynamics for Plasma Donors, United States. Emerg Infect Dis. 2021;27(11):2718-824.
67. 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(3):1111-8.
68. Choi YS, Lee J, Lee HW, Chang DY, Sung PS, Jung MK, et al. Liver injury in acute hepatitis A is associated with decreased frequency of regulatory T cells caused by Fas-mediated apoptosis. Gut. 2015;64(8):1303-13.
69. 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.
70. 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(3):927-36.
71. Jeong SH, Lee HS. Hepatitis A: clinical manifestations and management. Intervirology. 2010;53(1):15-9.
72. Radha Krishna Y, Saraswat VA, Das K, Himanshu G, Yachha SK, Aggarwal R, et al. Clinical features and predictors of outcome in acute hepatitis A and hepatitis E virus hepatitis on cirrhosis. Liver Int. 2009;29(3):392-8.
73. Ciocca M, Moreira-Silva SF, Alegría 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(8):711-5.

74. 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.
75. Bendre SV, Bavdekar AR, Bhave SA, Pandit AN, Chitambar SD, Arankalle VA. Fulminant hepatic failure: etiology, viral markers and outcome. Indian Pediatr. 1999;36(11):1107-12.
76. Ee LC, Shepherd RW, Cleghorn GJ, Lewindon PJ, Fawcett J, Strong RW, et al. Acute liver failure in children: A regional experience. J Paediatr Child Health. 2003;39(2):107-10.
77. Turk T, Al Saadi T, Sawaf B, Alkhatib M, Zakaria MI, Daaboul B. Progressive liver failure post acute hepatitis A, over a three-month period, resulting in hepatorenal syndrome and death. Gastroenterol Rep (Oxf). 2017;5(2):161-4.
78. 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(4):347-8.
79. Crowcroft NS, Walsh B, Davison KL, Gungabissoon U. Guidelines for the control of hepatitis A virus infection. Commun Dis Public Health. 2001;4(3):213-27.
80. 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(2):226-33.
81. Lin L, Verslype C, van Pelt JF, van Ranst M, Fevery J. Viral interaction and clinical implications of coinfection of hepatitis C virus with other hepatitis viruses. Eur J Gastroenterol Hepatol. 2006;18(12):1311-9.
82. 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(1):7-11.
83. Public Health England. Public health control and management of hepatitis A. London: PHE; 2017. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/727411/Public_health_control_and_management_of_hepatitis_A_2017.pdf.
84. De Paula VS. Laboratory diagnosis of hepatitis A. Future Virology. 2012;7(5):461-72.
85. Dos Santos G, Alves CR, Pinto MA, Amado Leon LA, Souza-Silva F. Detection of Antibodies against Hepatitis A Virus (HAV) by a Surface Plasmon Resonance (SPR) Biosensor: A New Diagnosis Tool Based on the Major HAV Capsid Protein VP1 (SPR-HAVP1). Sensors (Basel). 2021;21(9).
86. Nainan OV, Xia G, Vaughan G, Margolis HS. Diagnosis of hepatitis a virus infection: a molecular approach. Clin Microbiol Rev. 2006;19(1):63-79.
87. Rycroft JA, Mullender CM, Hopkins M, Cutino-Moguel T. Improving the accuracy of clinical interpretation of serological testing for the diagnosis of acute hepatitis a infection. J Clin Virol. 2022;155:105239.
88. Rasche A, Sander AL, Corman VM, Drexler JF. Evolutionary biology of human hepatitis viruses. J Hepatol. 2019;70(3):501-20.
89. 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(2):e0149642.
90. Lee GY, Kim WK, Cho S, Park K, Kim J, Lee SH, et al. Genotyping and Molecular Diagnosis of Hepatitis A Virus in Human Clinical Samples Using Multiplex PCR-Based Next-Generation Sequencing. Microorganisms. 2022;10(1).
91. Wang H, Chen W, Zhou W, Qiu F, Yin W, Cao J, et al. Exploration of a new hepatitis a surveillance system in Beijing, China: based on molecular epidemiology. BMC Infect Dis. 2022;22(1):22.

92. National Institute for Public Health and the Environment (RIVM). Typing protocol HAVNET VP1P2A. Bilthoven: RIVM; 2014. Available from: https://www.rivm.nl/documenten/typing-protocol-havnet-vp1p2a.
93. National Institute for Public Health and the Environment (RIVM). HAVNet Bilthoven: RIVM; 2019 [Available from: www.havnet.nl.
94. Haywood B, Tedder RS, Beebeejaun K, Balogun K, Mandal S, Andrews N, et al. Oral fluid testing facilitates understanding of hepatitis A virus household transmission. Epidemiol Infect. 2019;147:e105.
95. Liu JP, Nikolova D, Fei Y. Immunoglobulins for preventing hepatitis A. Cochrane Database Syst Rev. 2009(2):Cd004181.
96. 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(20-22):2878-83.
97. Lemon SM. Immunologic approaches to assessing the response to inactivated hepatitis A vaccine. J Hepatol. 1993;18 Suppl 2:S15-9.
98. 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(5):927-31.
99. World Health Organization. Hepatitis A Geneva: WHO; 2021 [Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-a#:~:text=Specific%20diagnosis%20is%20made%20by,may%20require%20specialized%20laboratory%20facilities.
100. Dunn R, Wetten A, McPherson S, Donnelly MC. Viral hepatitis in 2021: The challenges remaining and how we should tackle them. World J Gastroenterol. 2022;28(1):76-95.
101. Jacobsen KH. Globalization and the Changing Epidemiology of Hepatitis A Virus. Cold Spring Harb Perspect Med. 2018;8(10).
102. Kumar T, Shrivastava A, Kumar A, Laserson KF, Narain JP, Venkatesh S, et al. Viral Hepatitis Surveillance--India, 2011-2013. MMWR Morb Mortal Wkly Rep. 2015;64(28):758-62.
103. Friesema IH, Sonder GJ, Petrignani MW, Meiberg AE, van Rijckevorsel GG, Ruijs WL, et al. Spillover of a hepatitis A outbreak among men who have sex with men (MSM) to the general population, the Netherlands, 2017. Euro Surveill. 2018;23(23).
104. Pires SM, Jensen JD, Jakobsen L, Ethelberg S, Christensen T. Health and Economic Burden of Seven Foodborne Diseases in Denmark, 2019. Foodborne Pathog Dis. 2022.
105. Mangen MJ, Bouwknegt M, Friesema IH, Haagsma JA, Kortbeek LM, Tariq L, et al. Cost-of-illness and disease burden of food-related pathogens in the Netherlands, 2011. Int J Food Microbiol. 2015;196:84-93.
106. National Institute for Public Health and the Environment. Disease burden of food-related pathogens in the Netherlands, 2018 Bilthoven: RIVM; 2019 [Available from: https://www.rivm.nl/bibliotheek/rapporten/2019-0086.pdf.
107. Migueres M, Lhomme S, Izopet J. Hepatitis A: Epidemiology, High-Risk Groups, Prevention and Research on Antiviral Treatment. Viruses. 2021;13(10).
108. 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(3):379-85.
109. Lee YL, Chen GJ, Chen NY, Liou BH, Wang NC, Lee YT, et al. Less Severe but Prolonged Course of Acute Hepatitis A in Human Immunodeficiency Virus (HIV)-Infected Patients Compared With HIV-Uninfected Patients During an Outbreak: A Multicenter Observational Study. Clin Infect Dis. 2018;67(10):1595-602.

110. Cooksley WG. What did we learn from the Shanghai hepatitis A epidemic? J Viral Hepat. 2000;7 Suppl 1:1-3.
111. Xu ZY, Li ZH, Wang JX, Xiao ZP, Dong DX. Ecology and prevention of a shellfish-associated hepatitis A epidemic in Shanghai, China. Vaccine. 1992;10 Suppl 1:S67-8.
112. Hutin YJ, Pool V, Cramer EH, Nainan OV, Weth J, Williams IT, et al. A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. N Engl J Med. 1999;340(8):595-602.
113. Severi E, Verhoef L, Thornton L, Guzman-Herrador BR, Faber M, Sundqvist L, et al. Large and prolonged food-borne multistate hepatitis A outbreak in Europe associated with consumption of frozen berries, 2013 to 2014. Euro Surveill. 2015;20(29):21192.
114. Di Cola G, Fantilli AC, Pisano MB, Ré VE. Foodborne transmission of hepatitis A and hepatitis E viruses: A literature review. Int J Food Microbiol. 2021;338:108986.
115. Nasheri N, Vester A, Petronella N. Foodborne viral outbreaks associated with frozen produce. Epidemiol Infect. 2019;147:e291.
116. Tavoschi L, Severi E, Niskanen T, Boelaert F, Rizzi V, Liebana E, et al. Food-borne diseases associated with frozen berries consumption: a historical perspective, European Union, 1983 to 2013. Euro Surveill. 2015;20(29):21193.
117. Barrett CE, Pape BJ, Benedict KM, Foster MA, Roberts VA, Rotert K, et al. Impact of Public Health Interventions on Drinking Water-Associated Outbreaks of Hepatitis A - United States, 1971-2017. MMWR Morb Mortal Wkly Rep. 2019;68(35):766-70.
118. Williamson DA, Chen MY. Emerging and Reemerging Sexually Transmitted Infections. N Engl J Med. 2020;382(21):2023-32.
119. Cástková J, Benes C. Increase in hepatitis A cases in the Czech Republic in 2008 - an update. Euro Surveill. 2009;14(3).
120. Hrivniaková L, Sláciková M, Kolcunová S. Hepatitis A outbreak in a Roma village in eastern Slovakia, August-November 2008. Euro Surveill. 2009;14(3).
121. Bordi L, Rozera G, Scognamiglio P, Minosse C, Loffredo M, Antinori A, et al. Monophyletic outbreak of Hepatitis A involving HIV-infected men who have sex with men, Rome, Italy 2008-2009. J Clin Virol. 2012;54(1):26-9.
122. Dabrowska MM, Nazzal K, Wiercinska-Drapalo A. Hepatitis A and hepatitis A virus/HIV coinfection in men who have sex with men, Warsaw, Poland, September 2008 to September 2009. Euro Surveill. 2011;16(34).
123. Sfetcu O, Irvine N, Ngui SL, Emerson C, McCaughey C, Donaghy P. Hepatitis A outbreak predominantly affecting men who have sex with men in Northern Ireland, October 2008 to July 2009. Euro Surveill. 2011;16(9).
124. Tortajada C, de Olalla PG, Pinto RM, Bosch A, Caylà J. Outbreak of hepatitis A among men who have sex with men in Barcelona, Spain, September 2008-March 2009. Euro Surveill. 2009;14(15).
125. Frank C, Walter J, Muehlen M, Jansen A, van Treeck U, Hauri AM, et al. Major outbreak of hepatitis A associated with orange juice among tourists, Egypt, 2004. Emerg Infect Dis. 2007;13(1):156-8.
126. Sane J, MacDonald E, Vold L, Gossner C, Severi E. Multistate foodborne hepatitis A outbreak among European tourists returning from Egypt--need for reinforced vaccination recommendations, November 2012 to April 2013. Euro Surveill. 2015;20(4).
127. Beauté J, Westrell T, Schmid D, Müller L, Epstein J, Kontio M, et al. Travel-associated hepatitis A in Europe, 2009 to 2015. Euro Surveill. 2018;23(22).
128. Foster M, Ramachandran S, Myatt K, Donovan D, Bohm S, Fiedler J, et al. Hepatitis A Virus Outbreaks Associated with Drug Use and Homelessness - California,

Kentucky, Michigan, and Utah, 2017. MMWR Morb Mortal Wkly Rep. 2018;67(43):1208-10.
129. Herzog C, Van Herck K, Van Damme P. Hepatitis A vaccination and its immunological and epidemiological long-term effects - a review of the evidence. Hum Vaccin Immunother. 2021;17(5):1496-519.
130. Sun X, Wang F, Zheng H, Miao N, Yuan Q, Cui F, et al. The impact of expanded program on immunization with live attenuated and inactivated Hepatitis A vaccines in China, 2004-2016. Vaccine. 2018;36(10):1279-84.
131. 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(9389):1065-71.
132. Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. Clin Microbiol Rev. 2019;32(2).
133. 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(10):1629-34.
134. 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(3):169-77.
135. 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(12):3160-5.
136. Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines. A systematic review. Vaccine. 2012;31(1):3-11.
137. Wang X, Pan Y, Chen J, Luo J, Xi J, Ye C, et al. The excretion rate and stability of HAAg in human fecal samples after live attenuated hepatitis A vaccination. J Med Virol. 2020.
138. Mosley JW, Reisler DM, Brachott D, Roth D, Weiser J. Comparison of two lots of immune serum globulin for prophylaxis of infectious hepatitis. Am J Epidemiol. 1968;87(3):539-50.
139. Melgaço JG, Morgado LN, Santiago MA, Oliveira JM, Lewis-Ximenez LL, Hasselmann 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(32):3813-20.
140. Mayorga O, Bühler 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(10):1498-506.
141. 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(11):e0142297.
142. 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;13(11):2707-12.
143. Rosdahl A, Herzog C, Frösner G, Norén T, Rombo L, Askling HH. An extra priming dose of hepatitis A vaccine to adult patients with rheumatoid arthritis and drug induced immunosuppression - A prospective, open-label, multi-center study. Travel Med Infect Dis. 2018;21:43-50.
144. 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(17):1685-94.

145. Parrón I, Planas C, Godoy P, Manzanares-Laya S, Martínez A, Sala MR, et al. Effectiveness of hepatitis A vaccination as post-exposure prophylaxis. Hum Vaccin Immunother. 2017;13(2):423-7.
146. Kohl I, Nemecek V, Summerová M, Chlíbek R, Nad'ová K, Mináriková O. Long-term protective effect of post-exposure Havrix administration during viral hepatitis Type A outbreaks. Eur J Epidemiol. 2006;21(12):893-9.
147. Government of Canada. Hepatitis A vaccine: Canadian Immunization Guide Ottawa: Canada.ca; 2021 [26/02/2022]. Available from: https://www.canada.ca/en/public-health/services/publications/healthy-living/canadian-immunization-guide-part-4-active-vaccines/page-6-hepatitis-a-vaccine.html.
148. Nelson NP, Link-Gelles R, Hofmeister MG, Romero JR, Moore KL, Ward JW, et al. Update: Recommendations of the Advisory Committee on Immunization Practices for Use of Hepatitis A Vaccine for Postexposure Prophylaxis and for Preexposure Prophylaxis for International Travel. MMWR Morb Mortal Wkly Rep. 2018;67(43):1216-20.
149. Whelan J, Sonder GJ, Bovée L, Speksnijder A, van den Hoek A. Evaluation of hepatitis A vaccine in post-exposure prophylaxis, The Netherlands, 2004-2012. PLoS One. 2013;8(10):e78914.
150. Irwin DJ, Millership S. Control of a community hepatitis A outbreak using hepatitis A vaccine. Commun Dis Public Health. 1999;2(3):184-7.
151. 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(3):185-7.
152. Arce Arnáez A, Cabello Ballesteros L, Iñigo Martínez J. [Community outbreak of hepatitis A in a marginal population. Vaccination for its control and acceptance of the measure]. Aten Primaria. 2007;39(3):139-43.
153. Kelly D, O'Hare C, McKeown P, Cianci F, Doyle S. Detection and control of an ongoing international outbreak of hepatitis A among the Irish Traveller community beginning September 2020. Epidemiol Infect. 2022:1-13.
154. European Centre for Disease Prevention and Control. Hepatitis A outbreaks in the EU/EEA mostly affecting men who have sex with men – third update, 28 June 2017. Stockholm: ECDC; 2017. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/RRA%20hep%20A%20outbreak%20EU%20EEA%20in%20MSM%20third%20update%2028%20June%202017_0.pdf.
155. Mellou K, Sideroglou T, Papaevangelou V, Katsiaflaka A, Bitsolas N, Verykouki E, et al. Considerations on the current universal vaccination policy against hepatitis A in Greece after recent outbreaks. PLoS One. 2015;10(1):e0116939.
156. Regione Puglia. Bollettino delle Coperture Vaccinali nell'infanzia al 30 giugno 2018 Bari: Regione Puglia; 2018 [Available from: https://www.sanita.puglia.it/documents/20182/26673928/Bollettino_CV_Infanzia_Regione_Puglia_2007_giugno2018/25f0db7b-0852-4c94-9f93-699fe0109d4c?version=1.0&t=1548258463578.
157. Batalla J.; Urbiztondo L.; Borràs E.; Martínez M.; Cabezas C. Evolució del calendari de vacunacions sistemàtiques de Catalunya (període 198 0 - 2014). Butlletí Epidemiol Catalunya. 2014;35:15-29.
158. Asociación Española de Vacunología. Calendario de vacunación Ceuta y Melilla. Lleida: AEV; 2018 [Available from: https://www.vacunas.org/ceuta-y-melilla/.
159. Michaelis K, Poethko-Müller C, Kuhnert R, Stark K, Faber M. Hepatitis A virus infections, immunisations and demographic determinants in children and adolescents, Germany. Sci Rep. 2018;8(1):16696.
160. European Centre for Disease Prevention and Control. Hepatitis A virus in the EU/EEA, 1975-2014. Stockholm: ECDC; 2016. Available from:

https://www.ecdc.europa.eu/sites/default/files/media/en/publications/Publications/hepatitis-a-virus-EU-EEA-1975-2014.pdf.
161. Demicheli V, Carniglia E, Fucci S. The use of hepatitis a vaccination in Italy: an economic evaluation. Vaccine. 2003;21(19-20):2250-7.
162. Dimitrova M, Petrova G, Tachkov K, Bozhkova MK, Kamusheva M, Mitov K. Economic consequences of the vaccination against hepatitis A in the Bulgarian healthcare setting. Biotechnol Biotechnol Equip. 2014;28(2):366-71.
163. Diel R, Rappenhöner B, Schneider S. Cost-Effectiveness of Hepatitis A Immunization of Children and Adolescents in Germany. The European Journal of Health Economics. 2001;2(3):96-103.
164. Suijkerbuijk AW, Lugnér AK, van Pelt W, Wallinga J, Verhoef LP, de Melker HE, et al. Assessing potential introduction of universal or targeted hepatitis A vaccination in the Netherlands. Vaccine. 2012;30(35):5199-205.
165. Luyten J, Van de Sande S, de Schrijver K, Van Damme P, Beutels P. Cost-effectiveness of hepatitis A vaccination for adults in Belgium. Vaccine. 2012;30(42):6070-80.
166. Postma MJ, Bos JM, Beutels P, Schilthuis H, van den Hoek JA. Pharmaco-economic evaluation of targeted hepatitis A vaccination for children of ethnic minorities in Amsterdam (The Netherlands). Vaccine. 2004;22(15-16):1862-7.
167. Bhagey A, Foster K, Ralph S, Wardropper A, White C, Wholey V, et al. High prevalence of anti-hepatitis A IgG in a cohort of UK HIV-negative men who have sex with men: implications for local hepatitis A vaccine policy. Int J STD AIDS. 2018;29(10):1007-10.
168. World Health Organization. Strengthening surveillance of and response to foodborne diseases: a practical manual. Introductory module. Geneva: WHO; 2017.
169. World Health Organization. International Health Regulations (IHR). Geneva: WHO; 2005. Available from: http://apps.who.int/iris/bitstream/handle/10665/43883/9789241580410_eng.pdf?sequence=1.
170. European Commission. COMMISSION IMPLEMENTING DECISION (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Brussels: EC; 2018. Available from: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32018D0945&from=EN#page=10.
171. European Centre for Disease Prevention and Control. Surveillance systems overview for 2020 Stockholm: ECDC; 2022 [Available from: https://www.ecdc.europa.eu/en/publications-data/surveillance-systems-overview-2020.
172. European Centre for Disease Prevention and Control. ECDC activities on surveillance Stockholm: ECDC; 2022 [Available from: https://www.ecdc.europa.eu/en/about-ecdc/what-we-do/ecdc-activities-surveillance.
173. World Health Organization. Technical considerations and case definitions to improve surveillance for viral hepatitis. Geneva: WHO; 2016. Available from: https://apps.who.int/iris/bitstream/handle/10665/204501/9789241549547_eng.pdf.
174. World Health Organization. Technical considerations and case definitions to improve surveillance for viral hepatitis: technical report. Geneva: WHO; 2016 [Available from: https://apps.who.int/iris/bitstream/handle/10665/204501/9789241549547_eng.pdf.
175. Nicolay N, Le Bourhis-Zaimi M, Lesourd A, Martel M, Roque-Afonso AM, Erouart S, et al. A description of a hepatitis A outbreak in men who have sex with men and public health measures implemented in Seine-Maritime department, Normandy, France, 2017. BMC Public Health. 2020;20(1):1441.
176. European Centre for Disease Prevention and Control. Epidemic Intelligence Information System (EPIS). Stockholm: ECDC; 2021 [Available from:

https://www.ecdc.europa.eu/en/publications-data/epidemic-intelligence-information-system-epis.
177. European Centre for Disease Prevention and Control. EpiPulse - the European surveillance portal for infectious diseases. Stockholm: ECDC; 2022 [Available from: https://www.ecdc.europa.eu/en/publications-data/epipulse-european-surveillance-portal-infectious-diseases.
178. European Centre for Disease Prevention and Control. Field Epidemiology Manual Wiki - Outbreak Investigations Stockholm: ECDC; 2019 [Available from: https://wiki.ecdc.europa.eu/fem/Pages/Outbreak%20Investigations.aspx.
179. Gozlan Y, Bar-Or I, Volnowitz H, Asulin E, Rich R, Anis E, et al. Lessons from intensified surveillance of viral hepatitis A, Israel, 2017 and 2018. Euro Surveill. 2021;26(6).
180. La Rosa G, Libera SD, Iaconelli M, Ciccaglione AR, Bruni R, Taffon S, et al. Surveillance of hepatitis A virus in urban sewages and comparison with cases notified in the course of an outbreak, Italy 2013. BMC Infect Dis. 2014;14:419.
181. Pellegrinelli L, Galli C, Binda S, Primache V, Tagliacarne C, Pizza F, et al. Molecular Characterization and Phylogenetic Analysis of Enteroviruses and Hepatitis A Viruses in Sewage Samples, Northern Italy, 2016. Food Environ Virol. 2019;11(4):393-9.
182. European Centre for Disease Prevention and Control. Background: Constructing a Case Definition Stockholm: ECDC; 2012 [Available from: https://www.ecdc.europa.eu/sites/default/files/media/en/healthtopics/food_and_waterborne_disease/toolkit/Documents/tool-03-3-case-definitions-background.pdf.
183. Centers for Disease Control and Prevention. Lesson 1: Introduction to Epidemiology. Section 10: Chain of Infection. Atlanta: CDC; 2012 [Available from: https://www.cdc.gov/csels/dsepd/ss1978/lesson1/section10.html.
184. Dankwa EA, Donnelly CA, Brouwer AF, Zhao R, Montgomery MP, Weng MK, et al. Estimating vaccination threshold and impact in the 2017-2019 hepatitis A virus outbreak among persons experiencing homelessness or who use drugs in Louisville, Kentucky, United States. Vaccine. 2021;39(49):7182-90.
185. Bramer WM, Rethlefsen ML, Kleijnen J, Franco OH. Optimal database combinations for literature searches in systematic reviews: a prospective exploratory study. Syst Rev. 2017;6(1):245.
186. European Centre for Disease Prevention and Control. The European Surveillance System (TESSy). Stockholm: ECDC; 2022 [Available from: https://www.ecdc.europa.eu/en/publications-data/european-surveillance-system-tessy.
187. European Parliament; Council of the European Union. Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 april 2004 establishing a European Centre for disease prevention and control 2004 [Available from: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32004R0851.
188. Eurostat. Eurostat Luxembourg: Eurostat; 2022 [Available from: https://ec.europa.eu/eurostat.
189. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim JL, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. BMC Public Health. 2011;11:450.
190. Ludvigsson JF, Svedberg P, Olén O, Bruze G, Neovius M. The longitudinal integrated database for health insurance and labour market studies (LISA) and its use in medical research. Eur J Epidemiol. 2019;34(4):423-37.
191. World Health Organization. International Statistical Classification of Diseases and Related Health Problems (ICD) Geneva: WHO; 2022 [Available from: https://www.who.int/standards/classifications/classification-of-diseases.
192. European Parliament; Council of the European Union. Decision No 1082/2013/EU of the European Parliament and of the Council of 22 October 2013 on

serious cross-border threats to health and repealing Decision No 2119/98/EC. Official Journal of the European Union [Internet]. 2013.
193. Kirkwood BR, Sterne JAC. Essential medical statistics. Second edition. Malden: Blackwell Science; 2003.
194. Kuo CL, Duan Y, Grady J. Unconditional or Conditional Logistic Regression Model for Age-Matched Case-Control Data? Front Public Health. 2018;6:57.
195. Severi E, Booth L, Johnson S, Cleary P, Rimington M, Saunders D, et al. Large outbreak of Salmonella enteritidis PT8 in Portsmouth, UK, associated with a restaurant. Epidemiol Infect. 2012;140(10):1748-56.
196. Zenner D, Janmohamed K, Lane C, Little C, Charlett A, Adak GK, et al. The serotype case-case design: a direct comparison of a novel methodology with a case-control study in a national Salmonella Enteritidis PT14b outbreak in England and Wales. Epidemiol Infect. 2013;141(11):2346-53.
197. Montano-Remacha C, Ricotta L, Alfonsi V, Bella A, Tosti M, Ciccaglione A, et al. Hepatitis A outbreak in Italy, 2013: a matched case-control study. Euro Surveill. 2014;19(37).
198. Fitzgerald M, Thornton L, O'Gorman J, O'Connor L, Garvey P, Boland M, et al. Outbreak of hepatitis A infection associated with the consumption of frozen berries, Ireland, 2013--linked to an international outbreak. Euro Surveill. 2014;19(43).
199. Guzman-Herrador B, Jensvoll L, Einoder-Moreno M, Lange H, Myking S, Nygard K, et al. Ongoing hepatitis A outbreak in Europe 2013 to 2014: imported berry mix cake suspected to be the source of infection in Norway. Euro Surveill. 2014;19(15).
200. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine. 2010;28(41):6653-7.
201. World Health Organization. The Global Prevalence of Hepatitis A Virus Infection and Susceptibility: A Systematic Review. Geneva: WHO; 2010.
202. European Food Safety Authority. Tracing of food items in connection to the multinational hepatitis A virus outbreak in Europe. Parma: EFSA; 2014.
203. Bruni R, Taffon S, Equestre M, Cella E, Lo Presti A, Costantino A, et al. Hepatitis a virus genotypes and strains from an endemic area of Europe, Bulgaria 2012-2014. BMC Infect Dis. 2017;17(1):497.
204. Gillesberg Lassen S, Soborg B, Midgley SE, Steens A, Vold L, Stene-Johansen K, et al. Ongoing multi-strain food-borne hepatitis A outbreak with frozen berries as suspected vehicle: four Nordic countries affected, October 2012 to April 2013. Euro Surveill. 2013;18(17):20467.
205. Gossner CM, Severi E. Three simultaneous, food-borne, multi-country outbreaks of hepatitis A virus infection reported in EPIS-FWD in 2013: what does it mean for the European Union? Euro Surveill. 2014;19(43).
206. Stene-Johansen K, Tjon G, Schreier E, Bremer V, Bruisten S, Ngui SL, et al. Molecular epidemiological studies show that hepatitis A virus is endemic among active homosexual men in Europe. J Med Virol. 2007;79(4):356-65.
207. Gassowski M, Michaelis K, Wenzel JJ, Faber M, Figoni J, Mouna L, et al. Two concurrent outbreaks of hepatitis A highlight the risk of infection for non-immune travellers to Morocco, January to June 2018. Euro Surveill. 2018;23(27).
208. Zhang XS, Ong JJ, Macgregor L, Vilaplana TG, Heathcock ST, Mindlin M, et al. Transmission dynamics of the 2016-18 outbreak of hepatitis A among men who have sex with men in England and cost-effectiveness analysis of vaccination strategies to prevent future outbreaks. Lancet Reg Health Eur. 2022;19:100426.
209. European Centre for Disease Prevention and Control. EMIS 2010: The European Men-Who-Have-Sex-With-Men Internet Survey. Stockholm: ECDC; 2010 [Available from:

https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/EMIS-2010-european-men-who-have-sex-with-men-survey.pdf.
210. European Centre for Disease Prevention and Control. EMIS 2017: The European Men-Who-Have-Sex-With-Men Internet Survey. Stockholm: ECDC; 2017 [Available from: https://www.ecdc.europa.eu/sites/default/files/documents/European-MSM-internet-survey-2017-findings.pdf.
211. European Centre for Disease Prevention and Control. Hepatitis A outbreaks in the EU/EEA mostly affecting men who have sex with men – third update, 28 June 2017. Stockholm: ECDC; 2017.
212. Filia A, Rota MC, Grossi A, Martinelli D, Prato R, Rezza G. Are vaccine shortages a relevant public health issue in Europe? Results from a survey conducted in the framework of the EU Joint Action on Vaccination. Vaccine. 2022;40(13):1987-95.
213. WHO Regional Office for Europe. Assessing the magnitude and nature of shortages of essential medicines and vaccines: focus on the WHO European Region. Copenhagen: WHO/Europe; 2020.
214. Cella E, Golkocheva-Markova EN, Trandeva-Bankova D, Gregori G, Bruni R, Taffon S, et al. The genetic diversity of hepatitis A genotype I in Bulgaria. Medicine (Baltimore). 2018;97(3):e9632.
215. Mrzljak A, Bajkovec L, Vilibic-Cavlek T. Hepatotropic viruses: Is Roma population at risk? World J Gastroenterol. 2021;27(2):143-51.
216. Godoy P, Carmona G, Manzanares S, Jane M, Borràs E, Camps N, et al. Trends and risk factors of hepatitis A in Catalonia after the introduction of a hepatitis A+B vaccination programme. J Viral Hepat. 2018;25(9):1001-7.
217. Collier MG, Tong X, Xu F. Hepatitis A hospitalizations in the United States, 2002-2011. Hepatology. 2015;61(2):481-5.
218. 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(11):940-5.
219. Yoon JG, Choi MJ, Yoon JW, Noh JY, Song JY, Cheong HJ, et al. Seroprevalence and disease burden of acute hepatitis A in adult population in South Korea. PLoS One. 2017;12(10):e0186257.
220. Sobotka LA, Mumtaz K, Hinton A, Porter K, Conteh LF. National report on healthcare utilization and mortality in patients with hepatitis A infection in the United States. Public Health. 2022;207:113-8.
221. Kronen RJ, Haff M, Patwardhan V, LaSalvia M. Improving hepatitis A vaccination rates in hospitalised patients with cirrhosis: insights and lessons learnt. BMJ Open Qual. 2022;11(2).
222. Savage RD, Rosella LC, Brown KA, Khan K, Crowcroft NS. Underreporting of hepatitis A in non-endemic countries: a systematic review and meta-analysis. BMC Infect Dis. 2016;16:281.
223. Tosti ME, Longhi S, de Waure C, Mele A, Franco E, Ricciardi W, et al. Assessment of timeliness, representativeness and quality of data reported to Italy's national integrated surveillance system for acute viral hepatitis (SEIEVA). Public Health. 2015;129(5):561-8.
224. 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(4):596-602.
225. European Centre for Disease Prevention and Control. ECDC strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations – 2019–2021. Stockholm: ECDC; 2019.